MAR 29 1939

PERIODICAL ROOM
GENERAL LIBRARY
UNIV. OF MICH.

GROWTH

A JOURNAL FOR STUDIES OF DEVELOPMENT AND INCREASE

VOLUME II, NUMBER 4

PAPERS 64-76

DECEMBER, 1938 $3.50 A YEAR

GROWTH

A journal for the coordination of studies of increase and development as general properties of nature. Published by contributors and subscribers on a cooperative, non-profit, non-salary basis. The field of investigation is primary and work will be published in which the approach is through mathematics, physics, chemistry, genetics, embryology, philosophy and anthropology, just as will studies of development in its several expressions, and of mass increase and the part played therein by nutrition, vitamines and hormones.

Authors will receive *preprints* within a short period of acceptance for publication. Numbers of the journal will appear at irregular intervals. Subscription is $3.50 per volume annually (outside U. S. and Canada, $4.00), subscriptions to be sent to the *Managing-Editor*, Professor John Berrill, McGill University, Montreal, Canada. Single numbers $1.50. New subscriptions and renewals are entered to begin with the first issue of the current volume, one volume being issued per annum. Subscriptions must be for a volume or for separate numbers. (Beginning with Volume 3 the subscription rate will be $5.00 per volume.)

EDITORIAL BOARD

PHILOSOPHY: **L. K. Frank**
565 Park Avenue, New York

MATHS-DEMOGRAPHY: **H. L. Dunn***
U. S. Bureau Census, Washington, D. C.

CHEMICAL FACTORS:
F. S. Hammett (Chairman)
Lankenau Hosp., Res., Inst., Phila., Pa.
(address, Provincetown, Mass.)

GENETICS: **P. W. Gregory***
Univ. California, Davis, Calif.

EMBRYOLOGY-HUMAN GROWTH:
R. E. Scammon (Vice-Chairman)
Univ. Minnesota, Minneapolis, Minn.

ANTHROPOLOGY-HISTORY:
T. Wingate Todd
Western Reserve Univ., Cleveland, Ohio

PLANT GROWTH: **H. S. Reed***
Univ. California, Berkeley, Calif.

PROLIFERATION: **Philip R. White**
Rockefeller Inst., Princeton, N. J.

DIFFERENTIATION: **Leigh Hoadley**
Harvard Univ., Cambridge, Mass.

ORGANIZATION: **John Berrill**
McGill Univ., Montreal

NUTRITION: **S. Brody***
Univ. Missouri, Columbia, Mo.

PHYSICAL FACTORS:
D. M. Whitaker
Stanford Univ., Stanford, Calif.

HORMONES: **Leo Loeb**
Washington Univ., St. Louis, Mo.

BACTERIA-YEASTS: **Otto Rahn***
Cornell Univ., Ithaca, N. Y.

PROTOZOA: **D. M. Pace**
Johns Hopkins Univ., Baltimore, Md.

CHILD GROWTH:
Chas. B. Davenport
Carnegie Inst., Cold Spring Harbour, N.Y.

PATHOLOGICAL GROWTH:
Stanley P. Reimann
Lankenau Hosp. Res. Inst., Phila., Pa.

INTELLIGENCE AND LEARNING:
S. A. Courtis* (Secretary)
Univ. Michigan, Ann Arbor, Mich.

Corresponding Editors:

P. Boysen Jensen, *University of Copenhagen, Copenhagen*
Ivar Broman, *Lund, Sweden*
Luigi Castaldi, *Università di Cagliari, Cagliari, Italy*
A. Dalcq, *Université de Bruxelles, Brussels, Belgium*
E. Fauré-Fremiet, *College de France, Paris*
Julian S. Huxley, *Zoological Society, Regents Park, London*
J. Krizenecky, *Brno, Czechoslovakia*
Joseph Needham, *Caius College, Cambridge, England*
Yo. K. Okada, *Kyoto Imperial University, Kyoto, Japan*
G. Teissier, *Faculté des Sciences, Sorbonne, Paris*
A. H. Roffo, *Universidad de Buenos Aires, Buenos Aires, Argentine.*

* *Council*

For conditions and rates of publication see back inside cover.

T. WINGATE TODD

January 15, 1885–December 28, 1938

The early death of Doctor Todd at the age of fifty-three interrupts the work of a profound student of vertebrate growth whose contributions will assume increasing significance in the perspective of years to come. Todd early emphasized the importance of observing differentiation and maturation as contrasted with dimensional changes and he demonstrated, especially in bone growth, the orderly progress of growth, development, and maturation as revealed in non-dimensional alterations of configuration and texture. In his serial studies of human growth and development from birth through adolescence, he showed the unique value of longitudinal or consecutive observations on the same child, as disclosing the many sided but sequential process of growth and the individual's idiomatic progression through those sequences. The amazing range of his knowledge and his investigations was unified by his unwavering faith in the possibility of advancing human welfare through scientific study focussed upon the understanding of human growth and nurture. Not only as a scientist but as a rich human personality he will long be remembered by those who knew him.

L.K.F.

NOTICE

SYMPOSIUM ON GROWTH AND DEVELOPMENT

August 7–11, 1939

A SYMPOSIUM on growth and development will be sponsored by the editors of GROWTH and held at North Truro, Cape Cod, August 7–11, 1939. It is hoped that this will be the first of a series on Theoretical Biology. The occasion of this first meeting will be used to organize a society of persons interested.

THE main topic of the symposium is divided provisionally into four subtopics, Cellular Growth, Genes and Development, Chemical Differentiators, and Regenerative Growth. The primary object of the symposium is to explore the border line subjects of embryology and genetics, and as far as may be possible to relate such studies to the organismal concept of living forms. Proceedings of the symposium will be published as a supplement to GROWTH.

STUDIES OF MOULTING IN *CALLINECTES SAPIDUS* RATHBUN*

By

ELLEN H. GRAY AND CURTIS L. NEWCOMBE

Chesapeake Biological Laboratory and University of Maryland

(*Received October 17, 1938*)

In this study, moulting of the blue crab, *C. sapidus*, has been studied from a biometrical viewpoint. Four principal aspects of this process are treated, namely, time required to complete expansion, variation in percentage increment during moulting, sex variation in moulting, and number of moults after crabs have reached a width of 20 mm.

Several investigators have described, in a general way, the moulting process of certain Cancroid crabs. Among early references to moulting in this species are the account by Hay (1905), who includes a brief description of the more apparent phases of the change, and the somewhat longer treatment of the subject by Churchill (1918). Due to paucity of specimens studied, these workers were unable to establish valid conclusions in respect to the aforementioned specific aspects of this process. In a previous study, the extent of individual variation in the size of certain dimensions of *Callinectes sapidus* was determined and, also, the growth dimensional ratios that characterize the species (Gray and Newcombe, 1938). The present paper embodies the results of continued studies on the growth of this species, moulting having received major consideration.

It is a pleasure to acknowledge the kind cooperation in this work of Professor R. V. Truitt, Director of the Chesapeake Biological Laboratory, without whose assistance this study could not have been completed. Thanks are extended, also, to Captains Harvey Mister, Henry A. Kopp and Arthur Phillips; to Messrs. Mitchell Abbott and Ackley Tyler; and, particularly, to the firm of J. C. Lore and Company, of Solomons Island, for help in providing fresh material for examination.

METHODS OF HANDLING MATERIALS

The conclusions drawn from this study are based on measurements of 450 moulting crabs taken during July and August of the years 1935, 1936 and 1937.

Crabs that were ready to moult, "busters," were placed in a specially constructed crab float containing several compartments. Six hours after moulting both carapaces were measured in respect to width, length, and distance from the right eye to the right lateral spine. A vernier caliper, reading to a tenth of a millimeter, was used in all measurements. At the time of measuring, a record of the water temperature in the float was made. Only crabs that emerged in a healthy condition were used.

Specimens below 30 mm. in width (Groups II & III) were allowed to moult in finger bowls, kept under normal laboratory temperatures. Care was exercised,

* Contribution No. 24 of the Chesapeake Biological Laboratory.

while measuring, to keep all specimens out of the water for a minimum length of time in order to avoid loss of water from the body of the crab.

Data for male and female specimens were treated separately and, in both cases, 10 mm. interval groups were selected for study. Width, being the longest and most conspicuous dimension, was used as the basic index of size, in spite of the fact that length is known to be the most constant variable (Gray and Newcombe, 1938).

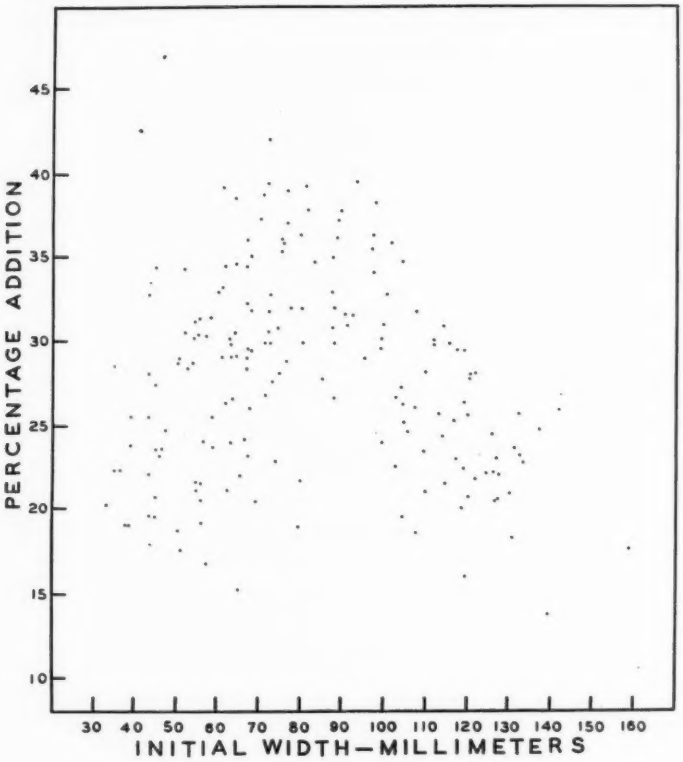


FIG. 1. Spot diagram showing individual variation in moulting of *C. sapidus* (males). Width range, 16.3 to 153.2 mm.

RESULTS

Individual variation constitutes a major problem in moulting studies within the group of decapod crustacea. *Callinectes sapidus* is not exceptional in this respect.

The time required for maximum expansion is highly variable, having been found in every case to be under six hours and sometimes as short as one hour

TABLE I

TIME REQUIRED FOR COMPLETION OF EXPANSION AFTER MOULTING IN *C. sapidus*: TOTAL NUMBER OF SPECIMENS OBSERVED—26

Hours Required	1+	2+	3+	4+	5+	
Percentage of Female Specimens, N=17	11.8	29.4	11.8	23.5	23.5	Total 100
Percentage of Male Specimens, N=9	22.3	44.4	33.3			Total 100

(Table I). Most of the growth was completed within the first hour, thus corroborating the findings of Churchill (1918). While maximum expansion occurs within six hours, measurements taken 48 hours after moulting, when the crab had become completely hard, showed a slight shrinkage in each dimension. This contraction is not sufficiently large to warrant consideration.

The magnitude of the spread that is a result of moulting is shown in Figure 1. Despite the apparent variation, it is indicated that a maximum size range exists with respect to expansion, namely, the width groups, 70 to 100 mm. This

TABLE II

MOULTING INCREMENTS OF *C. sapidus*

Group No.	Width Interval	No.	Mean Width		Abso. Incr.	% Addition	Mean Length		Abso. Incr.	% Addition	Mean Dist. Eye-to-spine		Abso. Incr.	% Addition
			Initial	Final			Initial	Final			Initial	Final		
						Females								
I	0– 9.9													
II	10– 19.9	8	16.76	18.76	2.00	11.93	8.92	10.53	1.60	17.93	6.15	6.93	0.78	12.79
III	20– 29.9	3	21.06	24.23	3.16	15.03	11.36	13.46	2.10	18.48	8.33	9.00	0.66	7.92
IV	30– 39.9	4	33.95	41.13	7.18	21.13	14.90	18.77	3.87	25.58	11.27	14.60	3.33	29.45
V	40– 49.9	29	45.31	57.93	12.62	27.86	22.13	28.05	5.92	26.75	16.47	21.77	5.32	32.17
VI	50– 59.9	52	55.38	71.90	16.52	29.82	26.83	34.04	7.22	26.88	20.71	27.04	6.33	30.59
VII	60– 69.9	66	65.02	87.52	22.50	34.59	31.23	40.22	8.99	28.78	24.65	33.46	8.81	35.55
VIII	70– 79.9	40	74.43	101.62	27.19	36.53	35.15	45.61	10.46	29.16	28.48	39.19	10.71	37.59
IX	80– 89.9	25	83.90	112.04	28.14	33.53	39.06	49.52	10.46	26.77	32.41	44.49	12.08	37.25
X	90– 99.9	14	94.56	131.33	36.77	38.87	43.16	54.02	10.86	25.15	36.96	51.90	14.94	40.43
XI	100–109.9	8	104.46	145.96	41.50	39.73	47.16	63.06	15.90	33.71	41.68	60.60	18.92	45.41
XII	110–119.9	5	116.34	157.58	41.24	35.44	50.34	64.24	13.90	27.65	50.16	74.32	24.16	48.16
XIII	120–129.9	3	122.00	165.13	43.13	35.35	55.00	67.96	12.96	35.75	48.93	67.56	18.63	38.08
XIV	130–139.9	2	131.50	176.25	44.75	34.03	57.25	69.90	12.65	22.09	54.00	70.90	16.90	31.29
						Males								
I	0– 9.9													
II	10– 19.9	5	17.62	20.10	2.52	14.38	9.20	11.04	1.84	20.00	6.54	7.80	1.26	19.26
III	20– 29.9	0												
IV	30– 39.9	7	36.23	44.63	8.40	23.18	17.50	22.00	4.50	25.71	12.70	15.67	2.97	22.57
V	40– 49.9	13	44.81	55.78	10.97	24.48	21.79	27.26	5.47	25.10	16.62	20.37	3.75	22.58
VI	50– 59.9	25	54.43	69.40	14.97	27.50	26.42	33.19	6.76	25.60	20.84	26.26	5.42	26.02
VII	60– 69.9	31	64.91	83.96	19.05	29.40	31.40	39.53	8.13	25.92	24.91	32.92	8.01	32.14
VIII	70– 79.9	24	74.05	98.12	24.07	32.51	35.00	44.50	9.50	27.11	28.74	38.64	9.91	34.45
IX	80– 89.9	17	84.98	112.97	27.99	32.93	39.65	50.08	10.43	26.32	33.72	45.62	11.90	35.26
X	90– 99.9	12	95.41	126.08	30.67	32.14	44.65	55.75	11.10	24.84	37.02	50.28	13.26	35.81
XI	100–109.9	15	104.85	133.27	28.42	27.10	47.89	58.60	10.71	22.35	41.99	54.26	12.27	29.21
XII	110–119.9	19	115.22	144.33	29.11	25.26	52.80	63.52	10.72	20.31	46.17	57.33	11.16	24.17
XIII	120–129.9	13	123.69	153.45	29.76	24.06	55.76	66.70	10.94	19.63	39.74	61.32	11.58	23.27
XIV	130–139.9	8	133.40	162.27	28.87	21.65	58.96	69.43	10.47	17.72	53.28	64.18	10.90	20.46
XV	140–149.9	1	149.10	175.50	26.40	17.70	65.40	77.50	12.10	18.50	60.50	70.90	10.40	17.19
XVI	150–159.9	1	153.20	190.40	37.20	24.47	66.50	79.00	12.50	18.80	61.50	77.00	15.50	22.30

peak is illustrated further by the maximum percentage additions obtained for male specimens of this range according to the method of grouping (Table II). The phenomenon is not so clearly shown in female crabs since their last moult is at an initial size of 100 to 110 mm, their maximum initial width range.

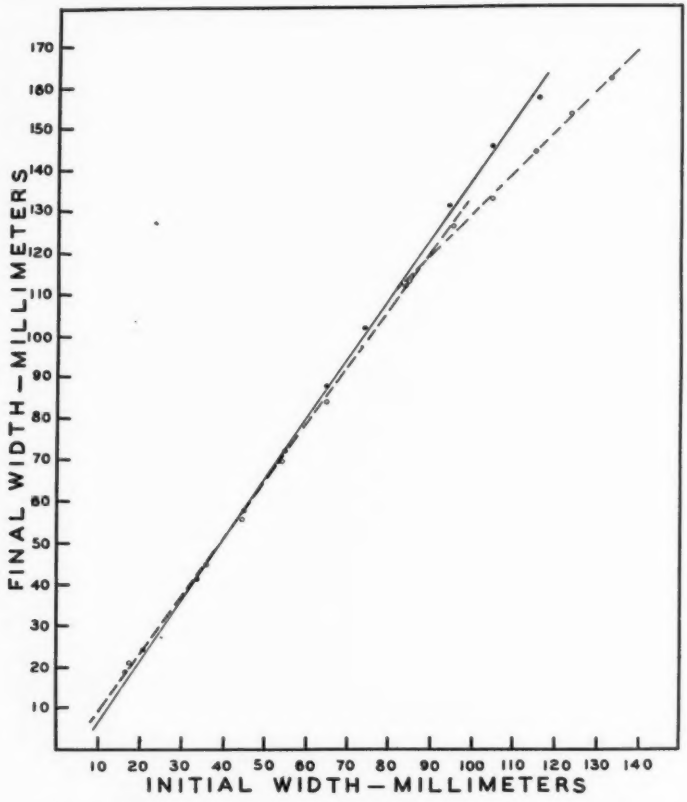


FIG. 2. Sex variation in the moulting of *C. sapidus* (width). Continuous line =females; dashed line=males. Range of initial widths—males, 16.3 to 139.8 mm. and females, 13.0 to 119.4 mm.

With respect to the three dimensions studied, the data indicate that the three series of moulting increments follow similar trends, starting in the male crabs as low as 14%, reaching a maximum of about 33% and falling off in the larger specimens to 21%. Female crabs are shown to have a moulting increment as low as 11%, attaining a maximum of 40% and dropping off to 35% in Group XII. In the three dimensions, the percentage increments for female specimens are slightly higher than those for males. Biometrical constants presented in Table III signify that, in most of the groups, the number of specimens is adequate to provide a reasonable degree of precision upon which to base conclusions.

A comparative study of moulting in male and female crabs has shown that the percentage increments of width do not differ by more than 3 percent until an initial width of about 89 mm. is reached. It appears that for female specimens, the ratio of initial width to final width is expressed by a straight line. In male crabs, two different ratios hold, the point of intersection occurring at an initial width of about 89 mm. (Fig. 2). The change in the slope is seen to be quite significant.

TABLE III

CONSTANTS OF THE FREQUENCY DISTRIBUTIONS OF THE DIMENSIONS—WIDTH, LENGTH AND DISTANCE FROM EYE-TO-SPINE IN *Callinectes*.

Group	No.	Range in mm.	Width Values		Length Values		Eye-to-spine Values	
			Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
Males								
I		0– 9.9						
II	5	10– 19.9	17.6±0.3	0.9±0.2	9.2±0.1	0.3±0.1	6.5±0.1	0.4±0.1
III	0	20– 29.9						
IV	7	30– 39.9	36.2±0.5	2.1±0.4	17.5±0.2	0.7±0.1	12.8±0.2	0.8±0.1
V	13	40– 49.9	44.8±0.3	1.5±0.2	21.8±0.2	1.0±0.1	16.6±0.2	1.0±0.1
VI	25	50– 59.9	54.4±0.3	2.6±0.2	26.4±0.2	1.6±0.3	20.8±0.2	1.5±0.1
VII	31	60– 69.9	64.9±0.3	2.7±0.2	31.4±0.2	1.5±0.1	24.9±0.2	1.8±0.2
VIII	24	70– 79.9	74.1±0.3	2.3±0.2	35.0±0.2	1.7±0.2	28.7±0.2	1.6±0.2
IX	17	80– 89.9	85.0±0.3	1.8±0.2	39.7±0.3	1.9±0.2	33.7±0.3	1.6±0.2
X	12	90– 99.9	95.4±0.7	3.6±0.5	44.7±0.5	2.4±0.3	37.0±0.6	3.0±0.4
XI	15	100–109.9	104.9±0.2	1.0±0.1	47.9±0.3	1.8±0.2	42.0±0.4	2.1±0.3
XII	19	110–119.9	115.2±0.5	3.6±0.4	52.8±0.3	1.9±0.2	46.2±0.2	1.5±0.2
XIII	13	120–129.9	123.7±0.5	2.5±0.3	55.8±0.3	1.7±0.2	49.7±0.3	1.6±0.2
XIV	8	130–139.9	133.4±0.8	3.3±0.5	59.0±0.5	2.0±0.3	53.3±0.4	1.6±0.3
Females								
I		0– 9.9						
II	8	10– 19.9	16.8±0.4	1.7±0.3	8.9±0.3	1.1±0.2	6.2±0.2	0.8±0.1
III	3	20– 29.9	21.1±0.6	1.5±0.4	11.4±0.5	1.2±0.3	8.3±0.4	1.1±0.3
IV	4	30– 39.9	34.0±0.8	2.5±0.6	14.9±0.5	1.5±0.4	11.3±0.5	1.4±0.3
V	29	40– 49.9	45.3±0.3	2.7±0.2	22.1±0.2	1.5±0.1	16.5±0.2	1.9±0.2
VI	52	50– 59.9	55.4±0.3	2.8±0.2	26.8±0.2	1.8±0.1	20.7±0.2	1.8±0.1
VII	66	60– 69.9	65.0±0.2	2.7±0.2	31.2±0.2	2.1±0.1	24.7±0.2	1.8±0.1
VIII	40	70– 79.9	74.4±0.3	2.6±0.2	35.2±0.2	2.1±0.2	28.5±0.2	2.0±0.2
IX	25	80– 89.9	83.9±0.1	0.6±0.1	39.1±0.2	1.5±0.1	32.4±0.2	1.6±0.2
X	14	90– 99.9	94.6±0.5	3.0±0.4	43.2±0.3	1.4±0.2	37.0±0.3	1.9±0.2
XI	8	100–109.9	104.5±0.8	3.3±0.6	47.2±0.7	3.0±0.5	41.7±0.8	3.5±0.6
XII	5	110–119.9	116.3±0.6	2.0±0.4	50.3±0.2	0.6±0.1	50.2±0.2	0.8±0.2
XIII	3	120–129.9	122.0±0.4	1.1±0.3	55.0±0.6	1.6±0.4	48.9±0.8	2.0±0.5

Figures 3 and 4 contrast the width and length dimensions in respect to their percentage increments during moulting. Until an initial width of about 44 mm. is reached, both male and female specimens, during moulting, increase less per unit of width than crabs with a corresponding length. In the case of large specimens, this relation is reversed. In other words, assuming the initial dimension (length or width) to be the same, the percentage increase in width exceeds that in length in the case of crabs greater than 44 mm. in width. A similar relation

holds with respect to the distance from eye to spine in male specimens (Fig. 5). Female crabs show a definite parallel condition with regard to the width and eye-to-spine dimensions (Fig. 3).

It is pointed out that male specimens exhibit a change in the ratio of growth at an initial width of about 89 mm. and at a length of about 44 mm. (Fig. 4).

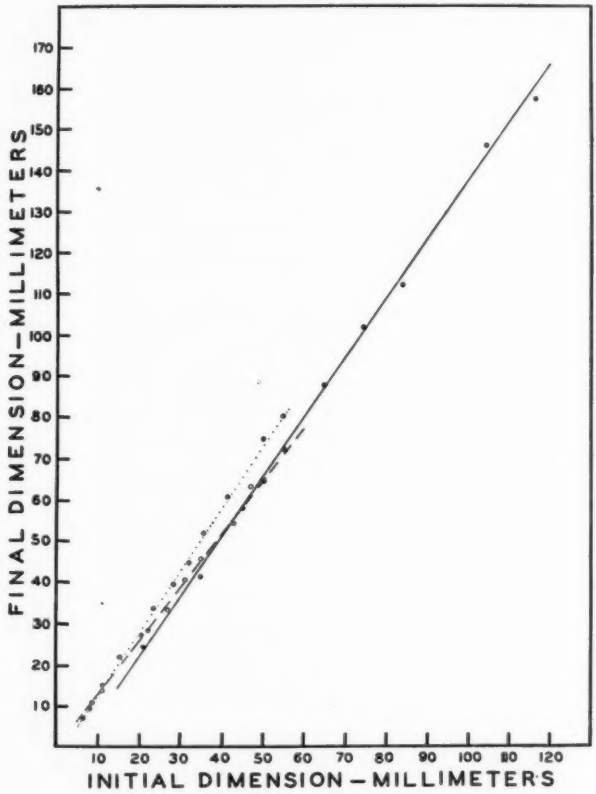


FIG. 3. Comparison of the width, length and eye-to-spine dimensions during moulting of *C. sapidus* (females). Continuous line = width; dashed line = length; dotted line = eye-to-spine. Range of initial length, 8.5 to 51.0 mm.; initial eye-to-spine, 5.0 to 51.0 mm.

The abrupt departures from the straight line relationship are by no means independent as is shown in Table II, Group X. Comparing the initial width and eye-to-spine growth ratio for males, it is seen that the ratio change takes place at a width of 89 mm. whereas the break in the eye-to-spine dimensional ratio occurs at about 34 mm. (Fig. 5). That these are related phenomena is shown by the agreement portrayed in Table II, Group IX.

On a basis of the data which have accrued from this study, it is possible to estimate the number of moults in crabs of 20 mm. or more in width. Using Fig. 2, it has been possible to estimate the probable number of moults by starting with a certain initial width e.g., 20 mm., and locating the corresponding final width, 22 mm., on the figure. Then, assuming this to be the next initial width, the corresponding final width after moulting, namely, 24.8 mm., is obtained. In a similar manner, the entire number of moults may be estimated for specimens ranging in width as low as 20 mm. (Fig. 6, Table IV).

TABLE IV

SHOWING METHOD OF ESTIMATING AVERAGE NUMBER OF MOULTS IN *C. sapidus* BY REFERENCE TO FIGURE 2

Specimens							
Males Initial Width 20 mm. moults		Females Initial Width 20 mm. moults		Males Initial Width 80 mm. moults		Females Initial Width 80 mm. moults	
from	*to*	*from*	*to*	*from*	*to*	*from*	*to*
20.0	22.0	20.0	22.9	80.0	106.0	80.0	108.2
22.0	24.8	22.9	26.9	106.0	135.2	108.2	148.8
24.8	28.5	26.9	32.6	135.2	164.2		
28.5	34.0	32.6	40.8	164.2	195.2		
34.0	41.6	40.8	48.4				
41.6	52.0	48.4	63.5				
52.0	66.8	63.5	84.2				
66.8	87.3	84.2	114.1				
87.3	126.5	114.1	156.9				
126.5	115.1						
155.1	184.0						
No. of moults, 11.		No. of moults, 9.		No. of moults, 4.		No. of moults, 2.	

Throughout the study of growth characteristics in *Callinectes*, the objective has been to present a clear picture of relations that typify this particular form. On a basis of equations obtained in a previous study (Gray and Newcombe, 1938), it is possible to verify the accuracy of the growth relations described here. Thus, a specimen 40 mm. in width possesses a length of 20.8 mm. (linear calculation) according to the equation $L=4.438-0.408W$ previously determined. This corresponding length dimension may be estimated indirectly. Referring to Fig. 2, it is seen that a crab 40 mm. in width reaches a width of 51 mm. on moulting. The length, according to the same width-length equation corresponding to a width of 51 mm., is 25.2 mm. Fig. 3 shows that a crab possessing a final length of 25.2 mm. had a length before moulting of 19.8 mm. It is seen that this estimated length compares very favorably with the calculated length of 20.8 mm. (Table V).

DISCUSSION

Interpretations of the data assembled during this study are based on average relationships determined for this species of *Callinectes*. The element of variation has been evaluated, at least in part, and data seriously limited by high

variability have not been included. This procedure seems to justify itself since it is impracticable to carry a significantly large number of individual crabs through the entire moulting process.

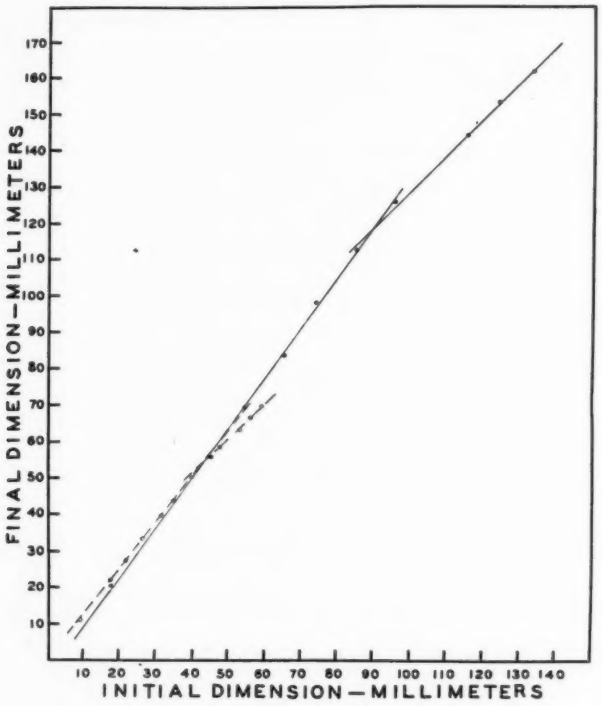


FIG. 4. Comparison of the width and length dimensions during moulting of *C. sapidus* (males). Continuous line = width; dashed line = length. Range of initial length, 8.9 to 62 mm.

Hay (1905) and Churchill (1918) have studied moulting in *Callinectes* of various sizes. Churchill concludes that the average increase in width during moult-

TABLE V

COMPARISON OF CALCULATED DIMENSIONS BASED ON LINEAR EQUATIONS AND MOULTING INCREMENTS IN *C. sapidus*

Sex	Basic Dimension Width in mm.	Corresponding Dimensions			
		Length		Eye-to-spine	
		Linear Calculation	Moulting Calculation	Linear Calculation	Moulting Calculation
Females	40	20.8	19.8	13.6	14.5
	110	47.8	49.0	44.1	43.2
Males	40	21.0	20.0	14.5	14.6
	110	49.8	51.0	43.8	43.8

ing is 32.7%, that the increase from the first crab stage to the second is over 50% and that at subsequent moultings the increase varies from 22 to 44%. The results are based on less than one hundred observations, many of which were made

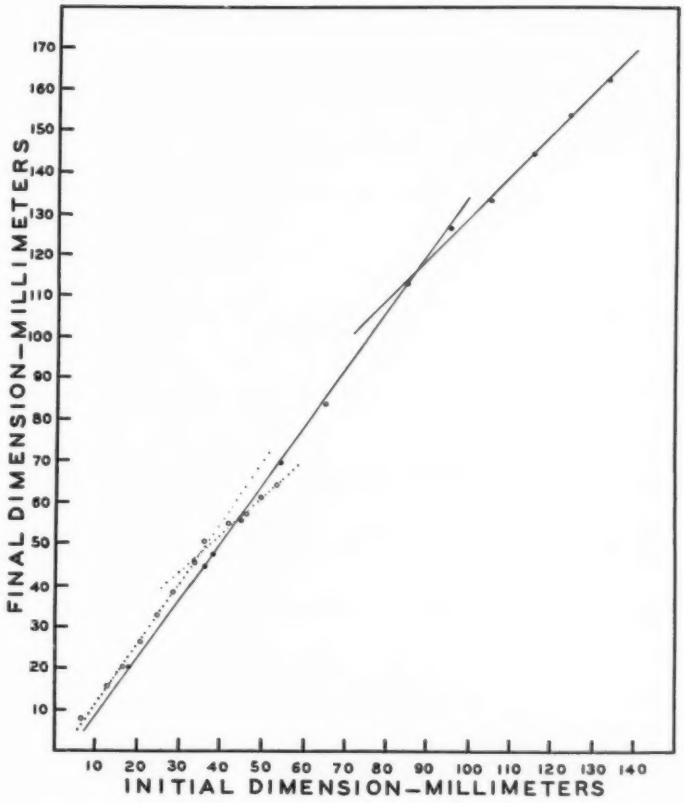


FIG. 5. Comparison of width and distance from eye-to-spine dimensions during moulting of *C. sapidus* (males). Continuous line = width; dotted line = eye-to-spine. Range of initial distance from eye-to-spine, 6.0 to 55.4 mm.

on the same specimen, taken at irregular intervals of width. In these studies, Churchill's findings, in so far as their general implications are concerned, have been found to be essentially true. The present work, based on the examination of 450 crabs, has yielded a more exact picture of the relation of initial size to the extent of moulting as well as of the variations throughout life in respect to the ratio of the initial to the final dimensions (width, length and distance from eye-to-spine).

Comparing the three dimensions measured, it is shown in Table II that each one follows a similar trend starting as low as 11%, reaching a peak of 39% in

Group XI. and descending to approximately 34%. There appears to be some variation due to sex. At present, it is impossible to explain the variation that seems to exist in specimens which have not attained the peak of expansion. Female crabs apparently do not moult after reaching maturity, at which size the peak of moulting is reached. In the case of male specimens, moulting continues beyond the peak (Fig. 6). Examination of Figure 6 shows that on the average male crabs undergo more moults than female specimens of similar initial size. Male specimens with an initial width of 20 mm. undergo 11 additional moults as compared to 9 in female crabs of corresponding initial size. The curve in Figure 6, of course, represents only an estimation of the number of moults,

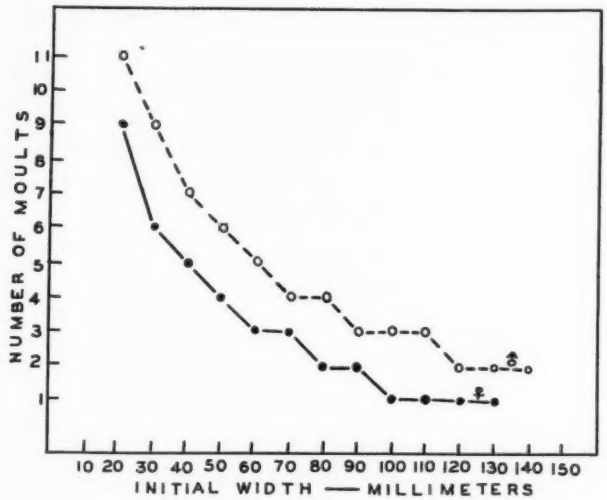


FIG. 6. Relationship between initial width of *C. sapidus* and the estimated number of moults. Continuous line=females; dashed line =males. Range of initial width, 20 to 140 mm.

nevertheless, on a basis of the numerous observations reported here, it does indicate the type of growth that characterizes this form.

With regard to the time required for completion of expansion, it should be noted that water temperature and the health of the crab are factors that largely determine variability of expansion. Although only 26 observations were made with respect to the time necessary for completing expansion, it appears quite definite that female crabs about to become mature require a longer time to expand than do the male and immature females. The female specimens, after their last shedding, require a longer time in which to complete growth, evidently because of greater modifications of the exoskeleton, wherein the triangular apron becomes rounded, the spines straightened and lengthened and the body excessively thickened.

In both sexes, the three dimensions treated exhibit a similar peak of expansion, with respect to size. The peak in the females corresponds with the size at which sexual maturity is reached, and constitutes one reason for believing that the peak in the males also indicates the size of sexual maturity. Another reason in support of this contention is that a break occurs at this point in the ratio of initial width to final width (Fig. 2), and it would suggest that a critical point in the life of the male crab has been reached at this time. Furthermore, considering external modifications, it is observed that at this particular size the spines tend to curve anteriorly, showing a definite modification in the exoskeletal structure. This hypothesis is further substantiated by the particular points where breaks occur in the moulting ratios for length and also for the eye-to-spine dimensions. For crabs having a length of about 44 mm. and an eye-to-spine dimension of 34 mm., the corresponding width is 89 mm. which is the point where the so-called break in the moulting ratio occurs.

In concluding this discussion of biometrical relations that typify crabs of this species, it should be recalled that significance is attributed here not to individual ratios and variations as occurring in living specimens observed during successive moultings, but, rather, to the establishment of group ratios and group variations of known numerical values. The statistical constants of frequency distributions within the groups are given in Table III. Making use of the dimensional ratios previously determined (Gray and Newcombe, 1938) and employing the moulting ratios shown in Figures 2–6, it has been possible to show the degree of relative precision attained independently in these studies (Table V).

CONCLUSIONS

On a basis of extended field observations and measurements before and after moulting of several growth dimensions of *Callinectes sapidus* of the Chesapeake Bay, the following conclusions have been drawn:

1. Expansion is normally completed within six hours after the moulting process, the greater portion being finished by the end of the first hour.
2. Female crabs, in the stage following their last shedding, require a longer period of time to complete expansion than do males and immature females.
3. Specimens 16 mm. in width increase about 11% during moulting, whereas those 104 mm. in the same dimension increase 39%. Male specimens, which continue to moult after having reached sexual maturity, show a decrease accompanying increase in initial width.
4. The relationships between initial width, length and distance from eye-to-spine and the corresponding final dimensions are expressed by a straight line in the case of female crabs.
5. In males, the relationship between the initial and final sizes of these three dimensions changes conspicuously at the initial width of about 89 mm. This is considered to be the size at which sexual maturity occurs in the male crab.
6. The number of moults of male crabs above 20 mm. in width is estimated to be 11, being two more than the number for female specimens of the same initial size.

LITERATURE CITED

CHURCHILL, E. P., 1918. Life History of the Blue Crab: *Bull. Bur. of Fish.*, Vol. 36, pp. 93–128, 1917–18

GRAY, ELLEN H. and NEWCOMBE, CURTIS L., 1938. Relative Growth of Parts of the Blue Crab, *Callinectes sapidus* Rathbun—GROWTH, Vol. 2, No. 3, pp. 235–246, 1938.

HAY, W. P., 1905. Life History of the Blue Crab, *Callinectes sapidus. Rep. U. S. Bur. of Fish.*, 1904, pp. 397–413, Washington, 1905.

A CORRELATION BETWEEN SULFHYDRYL, MITOSIS, AND CELL GROWTH IN LENGTH IN ROOTS OF PHASEOLUS VULGARIS

By

FREDERICK S. HAMMETT

With the technical assistance of

SIDNEY S. CHAPMAN

The Marine Experimental Station of The Lankenau Hospital Research Institute
North Truro, Massachusetts

Aided by a Grant from the Blanche and Frank Wolf Foundation, Inc.

(*Received for publication November 17, 1938*)

INTRODUCTION

Roots are the assumed portal of entry of most of those inorganic elements from which the plant builds the lifeless into the living. This is the becoming of growth (Hammett '36).

A first step in growth is proliferation, and proliferation is a dominant activity of the terminal region of roots. Inquiry into the possibility of relation between proliferation capacity of roots and capacity for transforming lifeless into living should therefore provide data useful in understanding how early growth processes are integrated.

In 1929 I reported confirmation of observations of Gola ('02) and Hopkins ('21) that SH occurs concentrated in the meristematic region of roots. This, and further studies, yielded the postulate that SH is a significant factor in cell proliferation (Hammett '29a).

In 1937 I reported with Reynolds that mashed root-tips of the common bean have the property of forming R.S-S.R from inorganic sulfate. This is reducible to R-SH by natural means or CN.

The principle that plant tissue changes inorganic sulfate to SH in organic combination was first experimentally indicated by Guthrie in 1933 and 1934. He found that as the sulfuric acid of potato tubers decreased the "glutathione" (R-SH) increased. The results were obtained on treatment with ethylene chlorhydrin.

Since this compound hastens the sprouting of potato tubers (Denny '26)—which is a proliferation expression—and since SH favors proliferation (Hammett '29a), evidently a part of the stimulation derives from a favoring action on SH production.

These observations place the two functions of transformation of lifeless into living and proliferation in the same region. They do not necessarily demonstrate an immediate association. Preliminary to such a demonstration knowledge is required of the relative intensity of these functions in the several parts of the growing plant.

A beginning in this direction is here made by study of the relation between the amount of SH and the two chief types of growth activity concerned in root elongation; viz., cell increase in number and cell increase in length.

I might point out that quite a little of the work being done in root growth is confused by the fact that many workers seem to forget that roots grow in length not only because the cells elongate, but also because the cells increase in number; and that since each of these separate growth activities is indubitably the expression of a different set of chemical reactions, so each activity indubitably has its own set of chemical determinants or "hormones."

PROCEDURE

An adequate supply of white beans (*Phaseolus vulgaris*) was allowed to sprout in autoclaved sphagnum wet with distilled water. From the roots so obtained there was cut the distal 1.0 to 1.5 mm of meristematic tissue. It is recognizable by its yellowish tinge. This served as control or proliferation tissue. Root lengths in any given experiment were essentially uniform.

For test material in one set of experiments the section immediately proximal to the control section was used. This represented an area of decreasing proliferation. In a second set the section was taken approximately mid-way from tip to stem. Here cell elongation dominated.

The cutting was done with small sharp glass chips to reduce oxidation and avoid contamination by metal. To reduce drying the sections were transferred as cut in groups of 20 or more to a moistened glass plate and then to a small weighing-bottle. One thousand control and 1000 test sections was usual for each trial. Each lot was weighed separately and then transferred to an agate mortar and pulped with 10 grams of dry washed and ignited fine sharp sand. Each mixture was washed into its own 15 ml centrifuge tube with 10 ml of distilled water. Each was centrifuged for 5 minutes at 1056 r.p.m. The supernatant fluid was then removed into another tube and similarly centrifuged.

The SH concentration of control and test was then immediately determined with nitroprusside under the rigorous control procedure described elsewhere (Hammett and Chapman '38).

If constancy in reagent concentration, order of admission of reagent, and time spacing, are observed, results can be obtained accurate to within plus or minus five percent in terms of simultaneous color development in cysteine standards. All reagents and standards must be freshly prepared. The first approximations should be made with a wide range of standards. The next approximation is made with a much narrower range. The final tracking down of the unknown concentration is between a close and decisive pair. The approximately 7 ml of extract yields ample material for analysis once experience in reading and rapidity of action are developed.

RESULTS

The table gives the milligrams of SH per 1000 roots, and per gram of root material. The data show comparison of the control meristematic section with the two test series.

The ratio of control to test SH is given for each experiment.

It is seen that the SH content of the terminal meristem is greater than that

of the contiguous tissue and much greater than that of the mid-section. The order of decreasing SH is thus—terminal meristem, contiguous meristem, mid-section.

The ratio of terminal to contiguous SH is well over twice that of terminal to mid-section SH.

The variability in absolute amount is greatest in the meristem tissue, somewhat less in the contiguous tissue, and very much less in the mid-section tissue. This holds on both the gram and the 1000 root basis. The difference is reflected

TABLE

THE AMOUNT OF SH IN THREE REGIONS OF ROOTS OF SPROUTING BEANS (*Phaseolus vulgaris*)

ROOT		Per 1000 Sections			Per Gram Root Substance		
		MERISTEM			MERISTEM		
Length cm.	Sections	Tip	Contig.	CM/TM	Tip	Contig.	CM/TM
		mg.			mg.		
0.8	1000	.0150	.0155	103.5	.0131	.0104	79.3
1.6	1000	.0150	.0135	89.7	.0136	.0092	67.6
2.9	1000	.0192	.0140	73.0	.0289	.0161	55.6
3.9	1000	.0140	.0135	96.3	.0304	.0202	66.7
4.2	1056	.0059	.0046	78.3	—	—	—
		Tip M.	Mid-Stem	MS/TM	Tip M.	Mid-Stem	MS/TM
2.6	925	.0126	.0057	48.9	.0181	.0058	32.2
2.8	1000	.0132	.0062	47.1	.0210	.0053	25.3
3.9	1000	.0098	.0052	52.6	.0148	.0044	29.4
4.1	1000	.0109	.0062	57.2	.0151	.0051	33.3
5.1	1000	.0111	.0057	51.2	.0165	.0053	31.9

in the ratio variability where that for the terminal-contiguous comparison is much greater than that for the terminal-mid-section relation. The latter value tends to maintain an equable order of magnitude.

DISCUSSION

These quantitative results place beyond question the highest SH concentration in the region of highest proliferation activity; the lower SH concentration in the region of lower proliferation activity; and the lowest SH concentration in the region where root cells are increasing in length rather than in number.

There is thus made quantitatively evident a positive correlation between cell increase in number and SH concentration; and a negative correlation between SH concentration and cell increase in length.

Consistent with this is the following:

Root-tip mitosis occurs with varying rather than uniform intensity. Therefore if SH is concerned therein it also should show considerable variability—which it does. And this variability—if it is dependent upon mitotic activity—should decrease with decrease in mitotic activity—which it does—being less in the contiguous meristematic tissue of lesser than terminal activity. And it should

be reduced to a negligible level where proliferation is negligible—as it does being insignificant in the relatively non-proliferating mid-section.

In the light of all that has heretofore been reported from this Institute the present findings constitute a quantitative confirmation from the chemical side of the postulate that the SH group is a naturally occurring chemical stimulus specifically related to cell increase in number as distinct from cell increase in size (Hammett '29a:b).

This distinction is real. Roots do grow in length because of cell increase in number as well as because of cell increase in length. Each property represents a separate growth activity and a separate set of chemical reactions. We know that SH is a determinant in one set—that responsible for increase in number; and it is probable that the auxins are determinants of the other—the set responsible for cell elongation.

I would here write a word of protest against the present too free use of the unqualified term "plant growth hormone" for this group of compounds. While the group may be a carrier of a common chemical radicle which acts as a stimulus to cell increase in length, this is expression of but a single growth activity. To dub the affecting chemical as *the plant growth hormone* implies that plant growth is solely cell elongation and that auxins are the only determining stimuli therefore.

I am sure that no chemically founded plant physiologist believes this; and of course any real student of growth knows that growth is not single but multiple, the summated expression of integrated developmental and incremental processes (Hammett '36). Confusion may be avoided if in the reporting of growth factors there is avoidance of loose usage of the term "growth." There should be adequate qualification. The auxins should not be blanketed as "plant growth hormones": they should be qualifiedly designated as "plant cell-elongation hormones." Just as SH is stated to be a proliferation stimulus (Hammett '29a), glycine a regeneration stimulus (Hammett '36a), pyrrolidone a differentiation factor (Hammett '38), and adenine of significance in organization (Hammett 38a).

It may come about with further analysis that SH will come to be known as the pre-mitotic proteolytic stimulus, while the auxins may be shown to be concerned specifically in cellulose formation. It has yet to be shown that SH is a direct participant in cell growth in size. There is no evidence that the auxins are directly concerned in cell increase in number. Each seems to be specific to its own activity. This is the expected on purely *a priori* grounds.

* * *

Now the critical student of growth may have accepted the parallelism between SH concentration and proliferation activity in roots as demonstrated. But if he is informed he has probably wondered why the SH concentration of the essentially non-proliferating mid-section tissue is so high.

The fact is that the SH found in the mid-section tissue does not represent save in very small degree the free SH as originally present therein. What is

found is largely the result of injury due to grinding. The evidence is two-fold.

When *intact* roots of sprouting beans are immersed in a saturated solution of $(NH_4)_2SO_4$ to which has been added a drop of one percent Na-nitroprusside and a drop of concentrated NH_4OH, color develops in maximum intensity in the region of maximum quantitative SH value. This fades away in the contiguous region of lesser quantitative SH content. It dies out long before the mid-section where no comparable reaction is found. The qualitative test shows there is essentially no free SH in the mid-section of intact roots. The qualitative data fit the proliferation picture exactly.

In my 1929 papers I reported that ". . . the SH reaction of root-tips is intensified by crushing. Moreover if . . . a scratch is gently made along the length (of a root) with a fine needle, an almost immediate production of a strong positive (nitroprusside) test is given following exactly the line of injury. These observations give decisive proof that SH is liberated by trauma."

It is from this that the SH found in the mid-section tissue of the reported analyses in this paper is derived. The deviation of SH amount from that to be expected on the basis of absence of proliferation activity is therefore apparent and not real.

As with the word "growth" so with the term "wound-hormone" is there careless usage. It would seem desirable to prove the compound alleged to be a wound hormone is actually produced by what may be called a wound. Prolonged, low-intensity, full ultra-violet irradiation can hardly be so designated. Here there is tissue disintegration. Such a breakdown is quite different from the liberation of loosely bound groups such as occurs when SH groups of native muscle proteins are activated by rise in pH (Mirsky '36).

The single fact that a given compound stimulates growth of tissue in bean-pods, or accelerates root length growth is no proof that it is a wound hormone. What is necessary is demonstration that the compound is produced by injury, not by disintegration, and that it actually stimulates cell increase in number.

The only compound so far uncovered which fulfills these requirements is the SH group. This does not mean that other compounds of similar characteristics do not exist. Probably there are several since wound healing is something more than cell increase in number, and of course cell increase in number has many steps, each with its own determinants (Hammett '36).

Here as elsewhere in the domain of growth investigation there is need for restricting rather than blanketing designations.

SUMMARY AND CONCLUSION

Quantitative analysis for SH of root sections of sprouting beans establishes a positive correlation between proliferation activity and SH concentration; and a negative correlation between SH concentration and root growth by cell elongation.

The order of increasing SH amount is mid-section, contiguous meristem, terminal meristem.

This is consistent with the postulate that SH is specifically associated with cell increase in number as distinct from cell increase in size.

REFERENCES

DENNY, F. E. 1926. Hastening the sprouting of dormant potato tubers. *Am. Jour. Bot.* **13:** 118–125.

GOLA, G. 1902. La Zofa e i suoi composti nell'economia delle pianti. Ricerche di fisologia vegetale. I. I composti solforati dei tissuti merestematici. *Malphigia* **16:** 368–392.

GUTHRIE, J. D. 1933. Change in the glutathione content of potato tubers treated with chemicals that break the rest period. *Contrib. Boyce Thompson Inst.* **5:** 331–350.

GUTHRIE, J. D. 1934. Metabolism of citric, sulfuric, and nitric acid in the potato tuber. An explanation for the high pH of the juice of tubers treated with ethylene chlorhydrin. *Contrib. Boyce Thompson Inst.* **6:** 247–268.

HAMMETT, F. S. 1929. Studies in the biology of metals. VI. The nature of the lead compound deposited in the growing root. *Protoplasma* **5:** 547–562.

HAMMETT, F. S. 1929a. The chemical stimulus essential for growth by increase in cell number. *Protoplasma* **7:** 297–322.

HAMMETT, F. S. 1929b. Cell division and cell growth in size. *Protoplasma* **7:** 535 540.

HAMMETT, F. S. 1936. *The Nature of Growth.* Science Press, Lancaster, Penn.

HAMMETT, F. S. 1936a. The regeneration favoring action of glycine. *Protoplasma* **27:** 65–68.

HAMMETT, F. S. 1938. 1-Hydroxyproline in developmental growth. *Growth* **1:** 291–298.

HAMMETT, F. S. 1938a. Developmental growth and nucleic acid components. VI. Adenine. *Growth* **1:** 55–62.

HAMMETT, F. S. and S. S. CHAPMAN. 1938. The quantitative unreliability of the nitroprusside test for SH and SS. *Jour. Lab. Clin. Med.* **24:** 293–298.

HAMMETT, F. S. and ARTHUR REYNOLDS. 1937. Disulfide from ammonium sulfate in the presence of mashed root-tips of *Phaseolus vulgaris. Science* **26:** 498–499.

A SIMPLE METHOD FOR ESTIMATING THE SURFACE AREA OF THE HUMAN BODY FROM BIRTH TO MATURITY

By

A. P. WEINBACH

From the Normal Child Development Study of the Department of Diseases of Children, Columbia University, and the Babies Hospital, New York

(*Received November 2, 1938*)

ACKNOWLEDGMENT

I am indebted to Dr. C. B. Davenport for the necessary anthropometry (measurement of thigh girths and heights) on the children (age birth to four years) brought to this department, and for kindly furnishing the heights, weights and thigh girths of a series of individuals (age four to 18 years) measured at Letchworth Village, Thiells, N. Y. I am further indebted to Dana Crandall for the major part of the statistical work; and to Kenneth Breeze for carefully tracing and lettering all the charts.

The widespread use of the surface area of the body as a means of evaluating the normalcy of heat production has caused investigators to search for a more precise method of determining the surface area, and to look either for a simple method of predicting surface area or for one which was readily usable by the

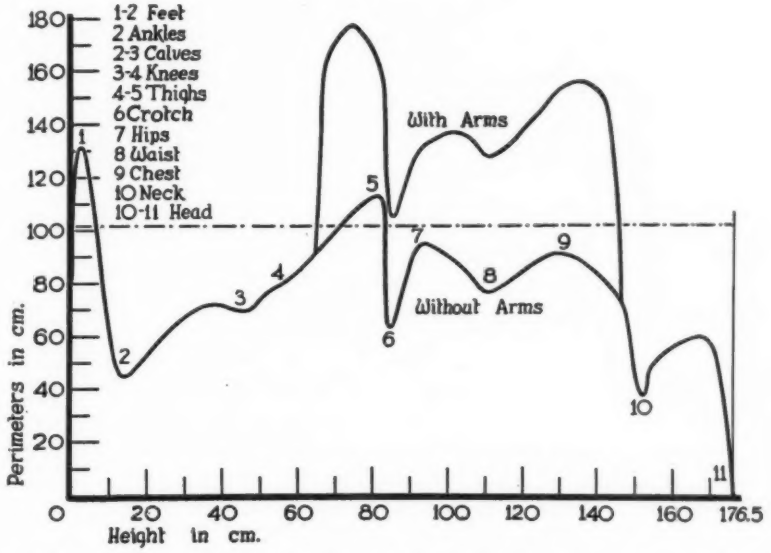


FIG. 1. Surface contour curve of adult male. The broken horizontal line passes through the mean height of the curve.

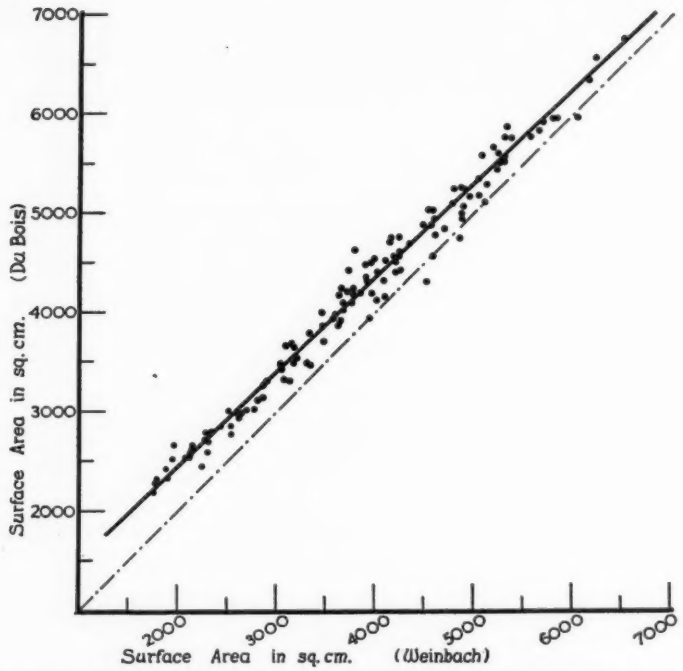


FIG. 2. Surface area calculated by the DuBois formula plotted against surface area obtained by multiplying twice the girth of the right thigh times the height for boys, birth to four years of age. The broken line shows where all points would fall, if the two methods gave identical results.

practitioner. Nomograms, charts and tables of complicated formulae relating surface area to height, weight, age, etc., have been proposed and used. It is the purpose of this paper to present a simple procedure for estimating surface area, and to compare the values obtained by this simple method with corresponding values computed from the DuBois equation. DuBois' formula was chosen (out of many) because it has been more extensively used than any other equation for the prediction of surface area of the human body.

When the surface contour of infants, children and adults was obtained by plotting the perimeters of serial sections through the body as ordinates against the height of the section from the soles of the feet as abscissae (1), it was noted that the mean height of the irregular curve, the area under which would be the total surface of the body was, in general, equal to the sum of the perimeters of the two thighs. That is, the ordinate corresponding to the mean height of the surface contour curve of the entire body cut the contour curve at the thighs. This is illustrated in Fig. 1 which is a surface contour curve of an adult male. The mean height, represented by the broken horizontal line, is obtained by dividing the area under the contour curve (curve including the arms) by the length of the base.

TABLE 1
MALES

Age years	Weight kilograms	Height cm.	Girth Rt. Thigh cm.	Surface Area Weinbach sq. cm.	Surface Area DuBois sq. cm.
0.01	3.529	53.0	16.6	1760	2184
0.02	3.161	52.3	15.2	1590	2064
0.04	3.927	54.0	16.5	1782	2317
0.06	3.147	51.7	15.2	1572	2043
0.07	4.126	55.6	16.9	1879	2416
0.07	5.295	54.5	18.0	1962	2648
0.09	4.635	54.8	17.8	1951	2512
0.10	3.756	54.1	16.4	1744	2276
0.11	4.486	57.3	18.6	2132	2590
0.11	4.614	57.5	20.0	2300	2583
0.12	4.324	57.5	18.0	2070	2526
0.13	5.089	57.1	20.2	2307	2693
0.14	4.337	54.6	20.5	2239	2436
0.15	4.636	58.7	18.2	2137	2641
0.15	4.968	58.5	19.4	2270	2713
0.15	5.313	60.2	21.0	2528	2850
0.17	5.444	59.0	20.7	2443	2838
0.18	4.820	56.0	19.0	2128	2595
0.19	5.936	61.2	22.6	2766	3023
0.21	5.330	57.7	22.0	2539	2768
0.21	5.833	61.3	22.0	2697	3046
0.22	5.882	60.0	22.0	2640	2969
0.23	4.991	60.2	19.2	2312	2775
0.25	5.670	60.4	21.7	2621	2937
0.27	5.657	62.2	20.2	2513	2997
0.28	6.719	64.3	24.3	3125	3303
0.29	7.283	65.5	25.5	3341	3464
0.30	6.718	63.4	22.6	2866	3269
0.33	6.237	61.8	22.8	2818	3110
0.34	6.677	64.5	22.5	2903	3302
0.36	7.385	66.7	24.0	3202	3531
0.39	7.256	65.6	23.2	3044	3462
0.42	6.704	64.6	24.1	3114	3311
0.42	7.272	66.0	24.0	3168	3481
0.42	8.679	70.4	28.0	3942	3933
0.48	7.995	68.0	25.6	3482	3704
0.50	7.116	65.7	23.2	3048	3438
0.52	7.385	69.8	22.1	3085	3649
0.59	7.286	71.0	22.2	3152	3673
0.59	8.193	70.4	25.8	3633	3879
0.61	8.406	71.0	25.6	3635	3904
0.62	8.363	68.0	24.5	3332	3775
0.67	8.363	73.5	23.5	3455	3994
0.67	9.246	70.0	27.0	3780	4024
0.69	8.731	70.0	25.4	3556	3927
0.69	8.902	71.3	26.1	4007	4012
0.69	10.734	76.2	30.0	4572	4559
0.74	8.575	74.8	24.5	3665	4089
0.75	9.327	72.5	28.2	4089	4152
0.76	9.186	71.8	26.2	3762	4087
0.77	9.086	70.6	26.0	3671	4018
0.79	11.141	78.6	30.8	4842	4737
0.82	8.817	75.4	24.1	3634	4161
0.84	9.228	75.0	24.3	3645	4226
0.84	9.682	72.0	27.5	3960	4188
0.86	9.483	72.5	26.0	3770	4172
0.86	11.793	78.1	30.0	4686	4830
0.90	8.959	75.7	24.5	3709	4202
0.94	9.809	74.9	26.0	3895	4333

TABLE 1—*Continued*

Age years	Weight kilograms	Height cm.	Girth Rt. Thigh cm.	Surface Area Weinbach sq. cm.	Surface Area DuBois sq. cm.
0.94	9.866	74.1	27.5	4076	4310
0.94	12.048	78.1	31.1	4858	4988
0.96	10.050	73.5	27.3	4013	4400
0.99	8.973	76.4	24.7	3774	4233
1.01	10.036	75.9	24.5	3719	4418
1.02	10.149	75.0	28.0	4200	4401
1.03	10.376	74.5	28.5	4247	4421
1.07	12.474	80.5	30.3	4878	5057
1.09	10.320	76.8	26.6	4086	4509
1.11	10.872	74.7	28.3	4228	4518
1.13	10.688	76.5	27.3	4177	4564
1.15	9.554	79.3	24.5	3886	4466
1.15	12.573	81.1	31.4	5093	5101
1.19	10.419	76.0	26.0	3952	4493
1.21	9.015	77.8	29.0	4512	4297
1.23	12.758	83.5	29.1	4860	5243
1.25	10.674	79.6	27.2	4330	4694
1.26	10.490	76.5	26.0	3978	4528
1.28	11.496	78.0	29.4	4586	4774
1.34	10.518	77.7	27.2	4227	4584
1.34	12.601	85.0	30.1	5117	5283
1.36	11.794	80.3	30.3	4866	4929
1.40	10.900	79.5	26.1	4150	4732
1.42	12.643	86.2	29.2	5034	5344
1.47	11.595	79.8	28.0	4469	4871
1.49	10.745	79.7	26.0	4144	4712
1.53	13.167	87.7	30.1	5280	5506
1.55	11.779	80.4	28.5	4583	4931
1.57	10.930	79.8	26.5	4229	4750
1.65	13.209	89.0	29.7	5287	5573
1.67	12.332	83.5	30.1	5027	5167
1.68	11.029	82.2	27.7	4554	4872
1.78	12.559	82.4	30.0	4944	5158
1.78	14.017	90.1	30.8	5550	5766
1.80	11.511	83.5	27.3	4559	5018
1.90	14.003	91.5	30.8	5636	5828
1.92	12.587	84.1	29.0	4884	5233
1.93	11.780	82.4	27.5	4532	5020
1.96	12.658	85.0	29.6	5032	5293
2.01	14.246	93.3	31.0	5785	5954
2.03	13.041	86.7	30.1	5219	5438
2.09	11.681	84.5	28.2	4766	5094
2.13	13.183	87.5	30.0	5250	5500
2.16	13.892	94.8	30.7	5821	5960
2.24	12.261	85.4	28.0	4782	5240
2.31	13.707	87.7	29.8	5227	5601
2.34	15.069	95.6	31.2	5965	6207
2.50	13.792	90.6	29.6	5364	5749
2.53	15.310	97.5	31.5	6143	6339
2.60	13.013	90.0	28.1	5058	5582
2.65	14.416	90.6	29.3	5309	5859
2.68	16.160	99.3	31.3	6216	6573
2.76	13.453	90.0	28.8	5184	5662
2.84	16.628	101.6	32.0	6502	6764
2.93	14.374	91.8	30.9	5682	5907
2.95	13.608	91.5	28.9	5289	5758
3.21	17.237	104.9	32.8	6881	7030
3.36	17.180	105.6	31.9	6737	7053
3.43	15.961	94.1	33.3	6267	6288
3.58	15.620	95.8	33.9	6495	6312

TABLE 1—*Continued*

Age years	Weight kilograms	Height cm.	Girth Rt. Thigh cm.	Surface Area Weinbach sq. cm.	Surface Area DuBois sq. cm.
4	17.12	105.0	30.6	6426	7014
4.5	18.48	104.1	32.0	6662	7200
4.5	19.96	107.6	33.0	7102	7621
5	18.48	106.0	32.4	6869	7295
5.5	18.60	109.6	31.0	6795	7495
5.5	15.65	103.8	28.0	5813	6695
6	15.54	101.1	29.4	5945	6549
6	16.10	106.4	27.0	5746	6899
6	17.69	109.9	29.7	6528	7353
6.5	16.10	103.5	29.0	6003	6762
7	18.09	111.4	29.3	6528	7495
7	19.73	115.2	31.0	7142	7968
7.5	18.60	113.1	30.8	6967	7667
8	20.19	115.4	31.0	7155	8057
8	22.45	121.2	33.0	7999	8734
8	25.17	126.4	34.0	8595	9451
8.5	23.02	123.0	33.2	8167	8922
9	24.95	127.3	33.6	8555	9465
10	26.99	131.4	36.2	9513	10014
10	30.17	132.5	37.0	9805	10563
11	38.44	140.8	43.5	12250	12234
11	31.50	137.0	38.7	10604	11022
12	39.46	145.7	43.6	12705	12684
12	40.71	145.8	45.3	13209	12859
12	44.68	151.9	44.9	13641	13783
12	36.51	142.1	42.9	12192	12051
12	32.89	141.7	39.6	11223	11504
12	29.71	134.2	36.0	9662	10591
13	43.55	151.1	45.9	13871	13580
13	46.61	150.7	48.1	14497	13951
13	54.09	156.6	50.2	15723	15282
13	39.35	146.7	44.9	13174	12731
13	36.29	148.0	40.8	12077	12379
13	33.91	139.1	39.0	10850	11499
14	43.43	153.7	44.0	13526	13740
14	50.46	157.4	46.5	14638	14892
14	63.62	167.0	51.0	17034	17155
14	44.34	151.6	46.8	14190	13717
14	42.53	154.8	43.3	13406	13682
14	34.70	142.9	39.0	11289	11841
15	49.90	159.4	46.5	14824	14957
15	60.22	163.6	51.8	16949	16510
15	65.66	172.3	51.1	17609	17784
15	47.85	155.6	46.9	14595	14438
15	48.08	161.6	45.0	14544	14871
15	37.99	148.1	40.6	12026	12629
16	56.67	166.3	46.5	15466	16281
16	69.74	176.5	53.5	18886	18561
16	53.75	163.4	47.6	15556	15718
16	55.57	167.5	47.2	15812	16230
16	41.73	152.9	41.5	12691	13451
17	65.55	172.7	50.8	17546	17801
17	78.81	178.5	57.3	20456	19718
17	56.25	168.7	47.5	16027	16399
17	60.67	169.7	49.0	16631	17007
17	44.79	158.5	43.2	13694	14228
18	49.78	163.3	45.3	14795	15206

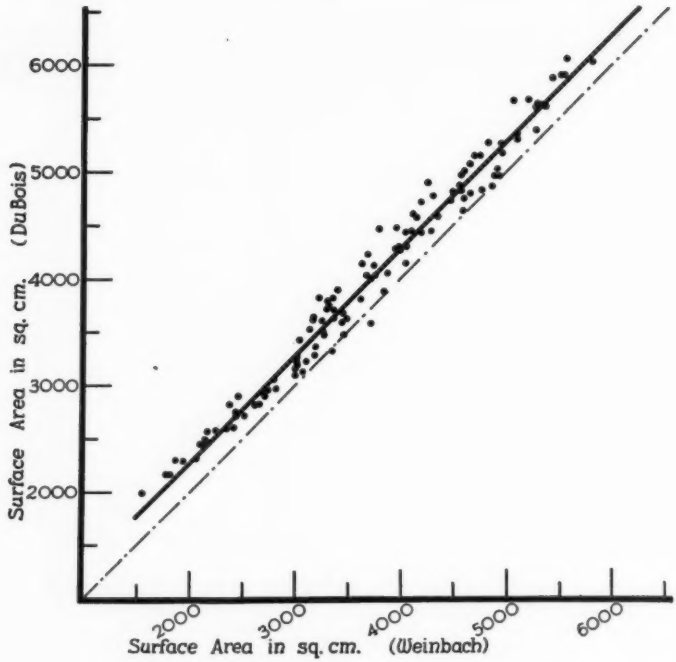


FIG. 3. Surface area calculated by the DuBois formula plotted against surface area obtained by multiplying twice the girth of the right thigh times the height for girls, birth to four years of age. The broken line shows where all points would fall, if the two methods gave identical results.

In consequence of the above observation, 369* estimations of surface area of individuals from birth to 18 years of age were obtained by multiplying the height of the individual by twice the perimeter of the right thigh. The thigh girths are taken horizontally at the most convex part of the thigh, usually at the base of each thigh at the gluteal fold. More precisely, the sum of the girths of the right and left thighs should be multiplied by the height, but to facilitate the technique twice the right thigh was used.

The results of determining the surface area by this simple method are compared in Figs. 2 to 6 with the surface as calculated by the DuBois formula,

$$A_d = 71.84 W^{0.425} H^{0.725}$$

where W is the weight in kilograms, H is the height in centimeters and A_d is the surface area in square centimeters.

Fig. 2 is a plot of the surface area obtained by the DuBois formula as ordinates versus the surface area obtained in square centimeters by multiplying the height in centimeters by twice the girth of the right thigh in centimeters, for

* The 369 estimations were made on 32 individuals from measurements taken at different ages.

TABLE 2
FEMALES

Age years	Weight kilograms	Height cm.	Girth Rt. Thigh cm.	Surface Area Weinbach sq. cm.	Surface Area DuBois sq. cm.
0.01	3.500	52.5	17.0	1785	2161
0.05	3.891	53.3	18.2	1940	2286
0.07	2.984	51.6	15.0	1548	1994
0.08	3.373	53.8	17.8	1915	2166
0.08	4.238	56.1	19.0	2132	2460
0.10	4.253	56.9	18.8	2139	2489
0.12	3.870	54.3	19.0	2063	2311
0.12	4.223	55.5	19.0	2109	2437
0.12	4.607	57.4	20.5	2353	2591
0.13	3.771	54.6	18.0	1966	2295
0.14	4.416	57.7	18.8	2170	2555
0.15	4.216	56.3	19.3	2173	2461
0.16	4.521	57.3	19.6	2246	2567
0.16	5.160	58.2	21.0	2444	2737
0.18	4.835	59.5	20.5	2440	2715
0.19	4.110	56.0	18.9	2117	2425
0.19	4.607	57.6	21.0	2419	2598
0.20	5.542	60.5	22.3	2698	2912
0.22	5.062	60.9	19.5	2375	2816
0.23	4.848	59.2	21.3	2522	2708
0.24	5.232	59.5	22.0	2618	2808
0.24	5.528	61.8	22.2	2744	2954
0.26	5.309	61.4	20.0	2456	2890
0.27	5.188	60.2	22.0	2649	2821
0.27	5.968	62.5	24.0	3000	3077
0.30	5.485	60.4	22.4	2706	2896
0.32	6.649	61.3	23.0	2820	2964
0.35	5.727	63.0	22.2	2797	3041
0.35	6.209	63.0	23.8	2999	3147
0.38	6.350	63.0	24.0	3024	3177
0.38	6.067	65.9	23.5	3097	3220
0.39	6.222	64.8	23.2	3007	3215
0.43	6.477	64.6	23.4	3023	3263
0.46	6.839	63.9	26.2	3348	3313
0.49	6.889	68.0	24.0	3264	3477
0.50	6.123	67.0	23.7	3176	3271
0.51	6.648	66.2	22.8	3019	3358
0.52	6.662	67.6	22.5	3042	3413
0.53	7.201	66.0	26.2	3458	3467
0.57	7.258	69.1	23.5	3248	3596
0.58	6.818	68.2	24.0	3274	3469
0.60	7.173	67.3	23.3	3136	3510
0.60	7.293	69.5	24.2	3364	3619
0.63	7.407	67.5	27.5	3713	3566
0.64	7.655	69.6	24.1	3355	3698
0.65	6.903	70.7	24.3	3436	3579
0.68	7.485	70.9	23.2	3290	3712
0.71	7.966	67.4	25.3	3451	3674
0.72	7.116	70.5	22.5	3173	3618
0.72	7.967	72.3	26.5	3832	3866
0.73	7.087	70.7	24.7	3493	3620
0.75	7.810	71.6	22.5	3222	3807
0.78	8.434	68.2	26.5	3615	3797
0.82	7.371	73.4	22.5	3303	3782
0.83	7.484	71.7	23.1	3313	3742
0.87	7.896	71.3	23.5	3351	3813
0.88	9.072	71.2	27.2	3873	4041
0.90	8.731	72.4	25.9	3750	4024
0.93	7.740	73.9	23.0	3399	3880
0.96	9.313	72.2	28.0	4043	4127

TABLE 2—*Continued*

Age years	Weight kilograms	Height cm.	Girth Rt. Thigh cm.	Surface Area Weinbach sq. cm.	Surface Area DuBois sq. cm.
0.99	8.463	72.5	25.6	3712	3975
1.00	8.618	73.7	24.0	3634	4133
1.01	8.604	73.0	25.1	3665	4023
1.07	8.846	74.0	25.2	3730	4111
1.14	9.043	75.3	25.5	3840	4202
1.15	9.526	78.3	25.8	4040	4420
1.18	8.944	76.3	24.1	3678	4223
1.19	10.234	75.0	28.5	4275	4416
1.22	9.242	75.6	26.3	3977	4254
1.23	9.312	75.9	26.7	4053	4280
1.26	8.859	77.8	25.3	3937	4265
1.30	10.773	77.5	29.5	4573	4622
1.31	10.050	80.0	25.7	4112	4592
1.32	9.214	76.6	25.9	3968	4289
1.33	9.879	76.8	26.7	4101	4426
1.37	9.539	79.0	24.0	3792	4451
1.39	9.810	78.0	25.3	3947	4463
1.42	10.518	80.3	26.0	4176	4695
1.42	9.496	78.3	26.7	4181	4414
1.42	11.255	79.1	29.4	4651	4779
1.45	9.936	80.1	27.0	4325	4574
1.49	10.717	82.0	27.8	4559	4805
1.53	11.354	80.3	30.2	4850	4850
1.54	10.801	79.8	28.7	4581	4727
1.56	9.980	79.6	26.0	4139	4562
1.56	10.376	81.6	27.3	4455	4722
1.65	10.702	82.1	29.0	4762	4806
1.67	11.510	82.0	30.0	4920	4953
1.68	10.462	82.0	26.8	4395	4756
1.68	10.688	83.4	27.2	4537	4858
1.76	10.575	82.6	27.1	4477	4803
1.78	11.865	82.9	28.0	4642	5057
1.81	11.471	84.7	27.1	4591	4987
1.82	11.057	83.9	29.1	4867	4950
1.87	10.816	83.3	25.5	4248	4879
1.89	11.865	83.9	29.5	4950	5101
1.93	11.355	86.7	27.0	4682	5127
1.94	11.511	85.1	28.8	4902	5088
1.95	11.029	84.2	27.1	4564	4958
2.04	11.624	88.3	27.9	4927	5248
2.09	11.568	86.6	28.5	4936	5164
2.10	11.581	85.9	27.5	4725	5136
2.19	11.780	89.8	28.3	5083	5342
2.24	12.135	88.8	29.6	5257	5367
2.26	12.035	87.5	29.0	5075	5291
2.37	12.418	92.2	29.0	5348	5569
2.39	11.893	87.4	27.5	4807	5260
2.42	12.504	90.6	29.0	5255	5515
2.56	12.645	92.6	28.0	5186	5630
2.57	12.646	91.5	29.0	5307	5581
2.65	12.871	91.2	29.0	5290	5610
2.72	13.524	94.7	29.1	5512	5888
2.73	12.900	93.0	29.0	5394	5695
2.83	12.800	92.5	27.3	5051	5654
2.88	13.312	95.0	28.5	5415	5862
2.92	13.638	97.4	29.0	5649	6030
3.10	13.580	94.6	29.2	5525	5893
3.25	13.892	95.9	30.2	5792	6010
3.82	13.991	99.6	31.0	6175	6196
3.97	14.317	101.0	30.3	6121	6279
4	13.95	95.0	30.1	5719	5979

TABLE 2—*Continued*

Age years	Weight kilograms	Height cm.	Girth Rt. Thigh cm.	Surface Area Weinbach sq. cm.	Surface Area DuBois sq. cm.
4.5	14.91	98.1	32.8	6435	6292
6.5	15.54	106.3	28.6	5974	6791
7.5	21.77	119.0	35.2	8378	8506
7.5	18.94	114.5	31.0	7099	7796
7.5	18.23	112.8	31.2	7039	7588
8	23.36	122.9	35.8	8800	8972
8	24.49	124.1	38.0	9432	9219
8	20.19	117.3	31.5	7390	8152
8.5	19.96	118.7	33.0	7834	8183
9	25.97	130.1	36.5	9497	9780
9	29.94	129.0	40.9	10552	10326
9	22.68	124.3	34.0	8452	8933
9	28.58	122.6	40.5	9931	9757
9.5	27.56	132.6	37.7	9998	10170
9.5	24.83	129.1	35.2	9089	9543
9.5	22.68	125.4	33.5	8402	8990
9.5	33.00	129.5	40.6	10515	10793
9.5	27.44	131.5	38.0	9994	10091
10	28.58	134.1	38.5	10326	10413
10	28.58	134.0	39.8	10666	10407
11	35.15	138.6	46.1	12779	11645
11	24.95	131.0	35.7	9353	9664
11	36.86	136.4	46.0	12549	11746
11	35.00	142.5	40.1	11429	11637
11	36.74	141.6	44.0	12461	12047
11	30.96	138.8	41.5	11520	11045
12	29.60	138.2	39.1	10807	10803
12	36.51	138.5	47.4	13130	11829
12	40.48	142.7	46.5	13271	12629
12	39.24	147.5	43.5	12833	12767
12	41.96	148.4	47.5	14098	13193
12	33.34	143.4	43.4	12447	11672
12	33.45	144.8	41.3	11960	11770
13	26.08	144.9	40.7	12085	10594
13	41.96	143.9	50.6	14563	12902
13	43.32	146.2	46.7	13655	13229
13	46.72	153.9	47.0	14467	14179
13	46.15	152.3	50.9	15504	13999
13	40.37	149.6	47.3	14152	13055
13	37.54	150.3	42.0	12625	12701
14	40.26	153.6	45.5	13978	13277
14	46.27	147.6	51.6	15232	13700
14	46.28	147.6	49.2	14524	13701
14	50.58	155.5	49.1	15270	14777
14	48.54	153.9	51.6	15882	14411
14	43.09	154.6	47.5	14687	13746
14	44.68	156.1	46.2	14424	14057
15	47.50	158.2	51.0	16136	14568
15	47.17	148.9	52.5	15635	13908
15	54.55	158.1	52.0	16442	15443
15	47.95	154.7	52.0	16089	14391
15	48.31	156.5	50.5	15807	14558
15	49.10	158.6	49.5	15701	14801
16	48.54	160.4	49.3	15815	14850
16	52.16	150.1	56.0	16811	14592
16	55.79	158.2	53.7	16991	15599
16	52.16	158.2	52.7	16674	15159
16	52.05	160.0	51.1	16352	15270
17	53.30	150.8	55.5	16739	14777
17	53.98	161.2	51.3	16539	15592
18	51.65	151.9	55.5	16861	14658

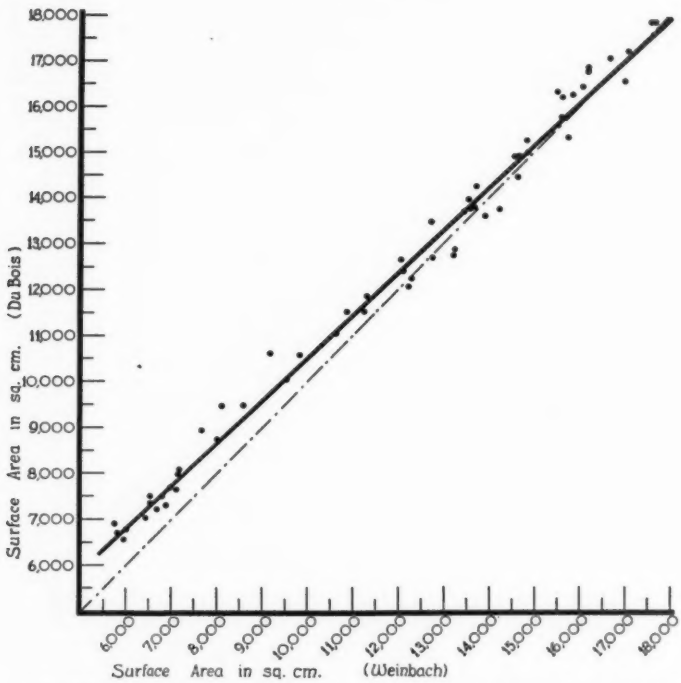


FIG. 4. Surface area calculated by the DuBois formula plotted against surface area obtained by multiplying twice the girth of the right thigh times the height for boys, four to 18 years of age. The broken line shows where all points would fall, if the two methods gave identical results.

boys from birth to four years of age. The coefficient of correlation between these two methods for estimating the surface area for boys in this age range is 0.99071. Fig. 3 is a similar plot for girls in the age range birth to four years with a coefficient of correlation of 0.99031. In Fig. 4 for boys from four to 18 years of age, the coefficient of correlation is 0.99578. Similarly, in Fig. 5, for girls from four to 18 years of age the coefficient of correlation is 0.98518 and in Fig. 6 for both sexes combined over the entire age range from birth to 18 years the coefficient of correlation is 0.99516. For boys alone over the entire age range from birth to 18 years the coefficient of correlation is 0.998124; and for girls alone over the entire age range the coefficient of correlation is 0.996245.

In general, the method of twice the right thigh times the height gives a lower value than that obtained by the DuBois formula: 2.57 percent lower for both sexes combined over the entire age range; 5.22 percent lower for boys alone over the entire age range; 0.18 percent higher for girls alone over the entire age range; 2.94 percent lower for boys alone from four to 18 years, 4.86 percent higher for girls alone from four to 18 years; 8.40 percent lower for boys alone from birth to 4 years; and 7.17 percent lower for girls alone from birth to four years of age.

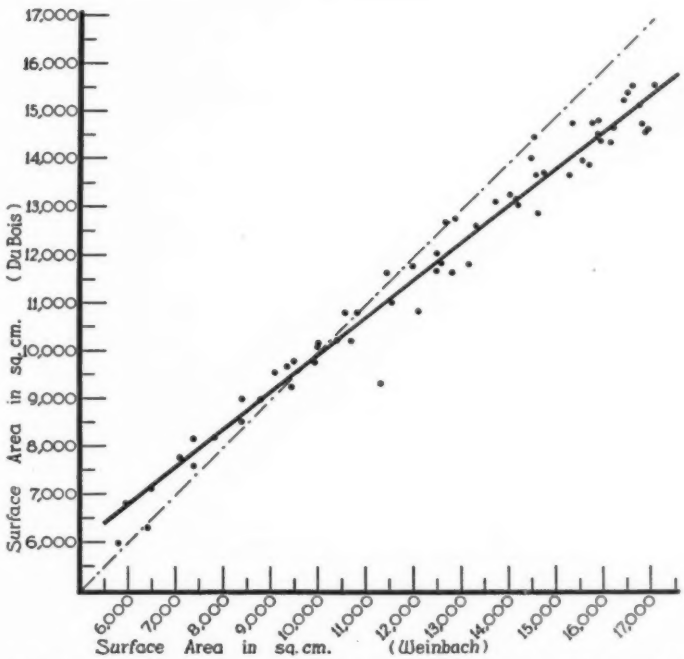


FIG. 5. Surface area calculated by the DuBois formula plotted against surface area obtained by multiplying twice the girth of the right thigh times the height for girls, four to 18 years of age. The broken line shows where all points would fall, if the two methods gave identical results.

Note: These percentage differences were obtained by

$$\frac{\overline{A}_d - \overline{A}_w}{\overline{A}_d} 100 \tag{1}$$

and are therefore misleading. The perfect regression equation between the two estimations of surface area (A_d and A_w) by the two different methods should pass through the origin and have a slope of unity.

$$A_d = A_w. \tag{2}$$

Actually, the regression equations obtained were of the form

$$A_d = mA_w + b. \tag{3}$$

The intersection of (2) and (3) occurs at

$$A_i = \frac{b}{1 - m} \tag{4}$$

which corresponds to an age t_i. Then below age t_i or surface area A_i, the method of twice the right thigh times the height gives a lower estimation of surface than

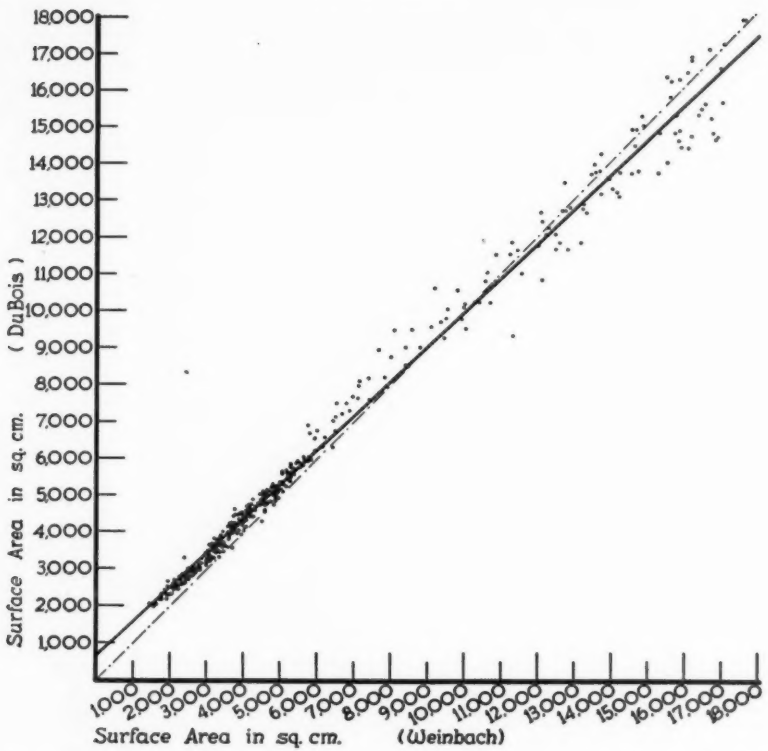


FIG. 6. Surface area calculated by the DuBois formula plotted against surface area obtained by multiplying twice the girth of the right thigh times the height for both sexes combined, birth to 18 years. The broken line shows where all points would fall, if the two methods gave identical results.

DuBois' formula, and above age t_i or surface A_i, the method of twice the right thigh times the height gives a higher estimation of surface than the DuBois formula.

A summary of the statistical constants for the curves in Figs. 2 to 6 is given below in which

A_w = the surface area obtained by multiplying twice the girth of the right thigh times the height.

A_d = the surface area obtained by the DuBois formula.

N = the number of observations.

S = the standard error of estimate of the A_d.

$\overline{A}_w$ = the arithmetic mean of the A_w.

$\overline{A}_d$ = the arithmetic mean of the A_d.

A_i = surface area at intersection of found regression line with line $A_d = A_w$.

Boys, birth to 4 years. Fig. 2

$r = 0.99071$
$N = 128$
$S = \pm 153.4$ cm.^{2}
$A_d = 0.94624A_w + 551.2$
$\overline{A}_d = 4131.7$
$\overline{A}_w = 3783.9$

$$\frac{S}{\overline{A}_d} 100 = 3.71\%$$

$$\frac{\overline{A}_d - \overline{A}_w}{\overline{A}_d} 100 = + 8.42\%$$

$A_i = 10,253$

Girls, birth to 4 years. Fig. 3

$r = 0.99031$
$N = 118$
$S = \pm 145.9$ cm.^{2}
$A_d = 0.99906A_w + 291.1$
$\overline{A}_d = 4011.8$
$\overline{A}_w = 3724.2$

$$\frac{S}{\overline{A}_d} 100 = 3.64\%$$

$$\frac{\overline{A}_d - \overline{A}_w}{\overline{A}_d} 100 = + 7.17\%$$

$A_i = 309,681$

Boys, 4 to 18 years. Fig. 4

$r = 0.99578$
$N = 60$
$S = \pm 335.3$
$A_d = 0.92596A_w + 1252.6$
$\overline{A}_d = 12,365.0$
$\overline{A}_w = 12,001.0$

$$\frac{S}{\overline{A}_d} 100 = 2.71\%$$

$$\frac{\overline{A}_d - \overline{A}_w}{\overline{A}_d} 100 = + 2.94\%$$

$A_i = 16,918$

Girls, 4 to 18 years. Fig. 5

$r = 0.98518$
$N = 63$
$S = \pm 442.7$
$A_d = 0.790596A_w + 2022.2$
$\overline{A}_d = 11,824.4$
$\overline{A}_w = 12,398.5$

$$\frac{S}{\overline{A}_d} 100 = 3.74\%$$

$$\frac{\overline{A}_d - \overline{A}_w}{\overline{A}_d} 100 = - 4.86\%$$

$A_i = 9,657$

Boys, birth to 18 years (curve not shown)

$r = 0.99812$
$N = 188$
$S = \pm 272.8$
$A_d = 0.981159A_w + 473.7$
$\overline{A}_d = 6759.37$
$\overline{A}_w = 6406.37$

$$\frac{S}{\overline{A}_d} 100 = 4.04\%$$

$$\frac{\overline{A}_d - \overline{A}_w}{\overline{A}_d} 100 = + 5.22\%$$

$A_i = 25,142$

Girls, birth to 18 years (curve not shown)

$r = 0.99622$
$N = 181$
$S = \pm 357.0$
$A_d = 0.885386A_w + 760.6$
$\overline{A}_d = 6731.12$
$\overline{A}_w = 6743.43$

$$\frac{S}{\overline{A}_d} 100 = 5.30\%$$

$$\frac{\overline{A}_d - \overline{A}_w}{\overline{A}_d} 100 = - 0.18\%$$

$A_i = 6,636$

Both sexes combined, birth to 18 years. Fig. 6

$$r = 0.99516$$
$$N = 369$$
$$S = \pm 421.8$$
$$A_d = 0.93186A_w + 621.6$$
$$\overline{A}_d = 6745.5$$
$$\overline{A}_w = 6571.7$$
$$\frac{S}{\overline{A}_d} 100 = 6.25\%$$
$$\frac{\overline{A}_d - \overline{A}_w}{\overline{A}_d} 100 = + 2.58\%$$
$$A_i = 9{,}122$$

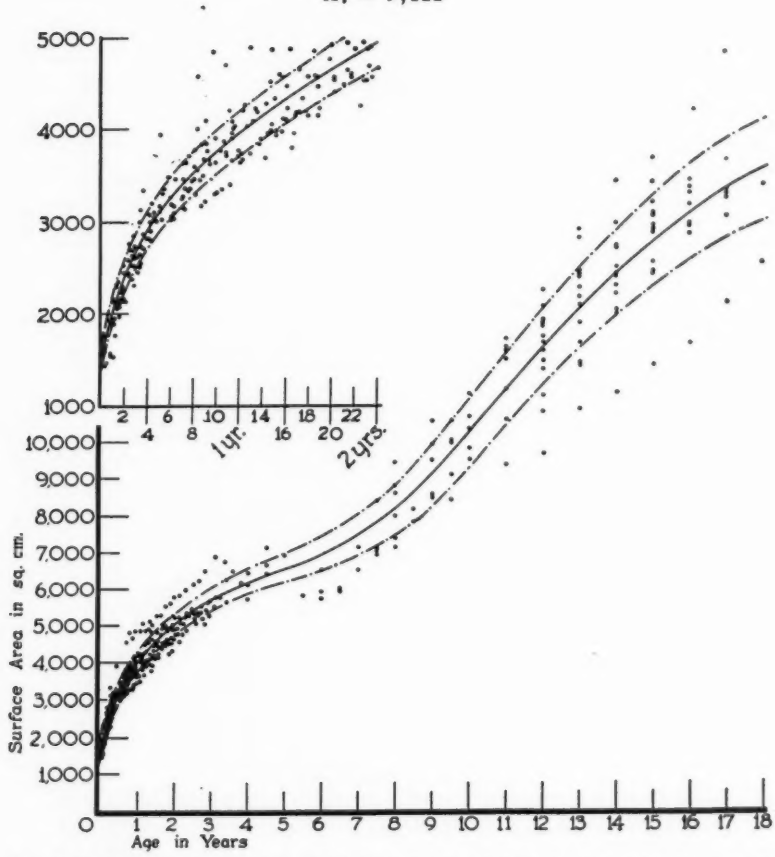


FIG. 7. Surface area obtained by multiplying twice the girth of the right thigh times the height plotted against age for both sexes combined. The curve of central tendency was drawn in by inspection to the mean at various ages, with the zone expected to include approximately the central two-thirds of the cases.

Fig. 7 is a plot of surface area by the method of twice the right thigh times the height as ordinates against age as abscissae for both sexes combined. The curve of central tendency was drawn in by inspection to the mean at various ages with zones expected to include approximately two-thirds of the cases. The inset shows the age range, birth to two years enlarged. This curve has the same shape as those given by Boyd (2) calculated from the Quetelet standards for height and weight and the Palmer standards for height and weight.

SUMMARY

The surface area of the normal human body from birth to maturity may be estimated by multiplying twice the girth of the right thigh times the height.

REFERENCES

1. Weinbach, A. P., 1938. Contour Maps, Center of Gravity, Moment of Inertia and Surface Area of the Human Body. *Human Biology*, **10**: 356–371.
2. Boyd, Edith, 1935. *The Growth of the Surface Area of the Human Body*. Univ. of Minn. Press.

ON A PROBLEM OF NATURAL INCREASE

By
W. M. DAWSON,
Bureau of Animal Industry; and
W. EDWARDS DEMING,
Bureau of Chemistry and Soils; Washington

(*Received November 28, 1938*)

1. Introduction. Problems of natural increase are from time to time of interest, possibly in long time planning where livestock or other growing colonies are involved. The following statement of an ideal problem was proposed by Sir Charles Ross of Washington and Dr. Ralph W. Phillips of the Bureau of Animal Industry, and its solution is here presented with the thought that it may be some value in answering other inquiries arising from problems of natural increase, even though the simple assumptions here taken are not sufficient to describe natural increase with all the complications that actually exist.

2. Statement of the problem. Starting with any number of newborn female lambs at the commencement of year 0, what will be the number of females in the flock at any time thereafter if each female gives birth to a lamb when she is two, three, four, five, six, and seven years old, half the lambs being female, and all sheep to be discarded at the end of their seventh year? In symbols

$$n_t = n_{t-1} + B_t - B_{t-8} \tag{1}$$

where n_t is the number of females in the flock during the t-th year, and B_t the number of females born at the beginning of the t-th year. The problem will be considered solved when n_t is expressed without approximation in terms of the original number n_0 in the flock.

It should be stated at the outset that the ideal solution just described was attained only to an approximation (Eq. 19) that holds within a few parts in 10 million after 40 years from the start, and with increasing accuracy beyond that. In addition, an exact formula (Eq. 9) was worked out to show n_t in terms of the number n_{t-1} of females living the year before, n_{t-2} two years before, and at other years previous. Another exact formula (Eq. 15) shows n_t as a function of the number n_{t-2} of females living two years previous, n_{t-4} four years previous, n_{t-6} six years previous—in other words, involving only every other year previous to t. The question proposed is therefore, after all, answered. The exact formulas (Eqs. 9 and 15) enable one to build up a table showing n_t for every year, as in Table 1, or every other year, as in Table 2. Beyond $t=40$, the simple approximation in Eq. 19 will give extremely good accuracy. Some variations of the problem are discussed in the last section.

3. The exact formulas. The assumption is that B_t, the number of females born at the beginning of year t, is half the number of females born two, three, four, . . . , and seven years previously; hence

$$B_t = \tfrac{1}{2}(B_{t-2} + B_{t-3} + \cdots + B_{t-7}). \tag{2}$$

It follows by substitution into Eq. 1 that

$$n_t = n_{t-1} + \tfrac{1}{2}(B_{t-2} + B_{t-3} + \cdots + B_{t-7}) - B_{t-8}. \tag{3}$$

By transposition Eq. 1 gives

$$B_t = n_t - n_{t-1} + B_{t-8} \tag{4}$$

whence by manipulating the suffixes it is clear that

$$\left.\begin{aligned} B_{t-8} &= n_{t-8} - n_{t-9} + B_{t-16} \\ B_{t-16} &= n_{t-16} - n_{t-17} + B_{t-24} \\ B_{t-24} &= n_{t-24} - n_{t-25} + B_{t-32} \\ &\vdots \end{aligned}\right\}. \tag{5}$$

These equations added together give

$$B_{t-8} = (n_{t-8} - n_{t-9}) + (n_{t-16} - n_{t-17}) + (n_{t-24} - n_{t-25}) + \cdots. \tag{6}$$

This value of B_{t-8} will presently be used in Eq. 3. First, however, the suffixes in Eq. 6 will be manipulated to get

$$\left.\begin{aligned} B_{t-2} &= (n_{t-2} - n_{t-3}) + (n_{t-10} - n_{t-11}) + (n_{t-18} - n_{t-19}) + \cdots \\ B_{t-3} &= (n_{t-3} - n_{t-4}) + (n_{t-11} - n_{t-12}) + (n_{t-19} - n_{t-20}) + \cdots \\ &\vdots \\ B_{t-7} &= (n_{t-7} - n_{t-8}) + (n_{t-15} - n_{t-16}) + (n_{t-23} - n_{t-24}) + \cdots \end{aligned}\right\}. \tag{7}$$

Addition of these equations gives

$$\begin{aligned} B_{t-2} + B_{t-3} + \cdots + B_{t-7} = {} & (n_{t-2} - n_{t-8}) + (n_{t-10} - n_{t-16}) \\ & + (n_{t-18} - n_{t-24}) + \cdots \end{aligned} \tag{8}$$

and we are now ready to substitute into Eq. 3. Replacing $B_{t-2} + \cdots + B_{t-7}$ by the value just written in Eq. 8, and B_{t-8} by what is given in Eq. 6, we find from Eq. 3 that

$$\begin{aligned} n_t = {} & n_{t-1} + \tfrac{1}{2}(n_{t-2} + n_{t-10} + n_{t-18} + \cdots) - (3/2)(n_{t-8} + n_{t-16} \\ & + n_{t-24} + \cdots) + (n_{t-9} + n_{t-17} + n_{t-25} + \cdots). \end{aligned} \tag{9}$$

This is the exact formula, mentioned earlier, giving the number of females n_t in terms of the number of females living in previous years, both even and odd. By this formula a table can be built up showing n_t for $t=0, 1, 2, 3, \cdots$, as in Table 1. For purposes of illustration, the original number of females, n_o, was arbitrarily taken as 100, as the table shows; and all numerical computations are relative to this number.

Another exact formula can be found, which skips every odd year previous to t. By lowering the suffixes one unit in Eq. 9 we see that

$$n_{t-1} = n_{t-2} + \tfrac{1}{2}(n_{t-3} + n_{t-11} + \cdots) - (3/2)(n_{t-9} + n_{t-17} + \cdots) + (n_{t-10} + n_{t-18} + \cdots). \tag{10}$$

This value of n_{t-1} substituted back into the right-hand side of Eq. 9 gives, after some collecting,

$$n_t = (3/2)(n_{t-2} + n_{t-10} + n_{t-18} + \cdots - n_{t-8} - n_{t-16} - n_{t-24} - \cdots) + \tfrac{1}{2}(n_{t-3} + n_{t-11} + n_{t-19} + \cdots - n_{t-9} - n_{t-17} - n_{t-25} - \cdots). \tag{11}$$

TABLE 1

TABLE SHOWING THE NUMBER OF FEMALES n_t LIVING AT THE BEGINNING OF EACH YEAR, OVER THE FIRST 20 YEARS, STARTING WITH 100 NEWBORN EWE LAMBS
Calculated according to Eq. 9.

Date 1st April	t	n_t	n_t/n_{t-1}
1900	0	100	...
1901	1	100	1
1902	2	150	1.5
1903	3	200	1.3
1904	4	275	1.375
1905	5	375	1.363636
1906	6	512.5	1.366666
1907	7	700	1.365850
1908	8	806.25	1.151786
1909	9	1106.25	1.372093
1910	10	1434.375	1.296610
1911	11	1887.5	1.315904
1912	12	2467.1875	1.307119
1913	13	3223.4375	1.306523
1914	14	4200.78125	1.303199
1915	15	5462.5	1.300353
1916	16	7139.765625	1.310712
1917	17	9337.890625	1.304217
1918	18	12200.5859375	1.306568
1919	19	15925.78125	1.305329
1920	20	20792.48046875	1.305586

To get rid of the indices in the last parenthesis dating back to odd years previous to t, we note from Eq. 9 that

$$n_{t-1} + n_{t-9} + n_{t-17} + \cdots = n_t - \tfrac{1}{2}(n_{t-2} + n_{t-10} + n_{t-18} + \cdots) + (3/2)(n_{t-8} + n_{t-16} + n_{t-24} + \cdots) \quad (12)$$

whereupon by slipping the index backward 2 years and then 8 years we find that

$$n_{t-3} + n_{t-11} + n_{t-19} + \cdots = n_{t-2} - \tfrac{1}{2}(n_{t-4} + n_{t-12} + n_{t-20} + \cdots) + (3/2)(n_{t-10} + n_{t-18} + n_{t-26} + \cdots) \quad (13)$$

$$n_{t-9} + n_{t-17} + n_{t-25} + \cdots = n_{t-8} - \tfrac{1}{2}(n_{t-10} + n_{t-18} + n_{t-26} + \cdots) + (3/2)(n_{t-16} + n_{t-24} + n_{t-32} + \cdots). \quad (14)$$

It will be noted that the indices on the right-hand sides of Eqs. 13 and 14

TABLE 2

COMPARISON OF n_t WITH THE PROJECTIONS OVER PERIODS OF TWO YEARS USING THE LIMITING RATIO $x = \text{Lim}\ n_t/n_{t-1} = 1.305650533$, BETWEEN $t=20$ AND $t=50$

Date 1st April	t	n_t (true value by Eq. 15)	$x^2 n_{t-2}$ (an approximation for n_t)	Error percent
1920	20	20792.48047	...	...
1922	22	35439.50196	35445.42621	0.0167165
1924	24	60419.94631	60414.54523	−.0089393
1926	26	103000.7202	102999.2911	−.0013875
1928	28	175587.4692	175587.7291	.0001480
1930	30	299331.3014	299328.0524	−.0010818
1932	32	510275.5988	510277.0481	.0002840
1934	34	869877.4788	869878.7098	.0001415
1936	36	1482900.828	1482900.419	.0000276
1938	38	2527934.842	2527935.614	.0000305
1940	40	4309429.540	4309429.461	−.0000190
1942	42	7346385.510	7346385.007	−.0000690
1944	44	12523554.68	12523554.65	−.0000003
1946	46	21349195.73	21349195.64	−.0000005
1948	48	36394471.77	36394471.54	−.0000007
1950	50	62042504.50	62042504.13	−.0000006

date back to even years previous to t, and that those on the left are just the ones occurring in the last parenthesis of Eq. 11. We are therefore provided with the substitution needed for Eq. 11. The result is

$$n_t = 2(n_{t-2} - n_{t-8}) - \tfrac{1}{4}(n_{t-4} + n_{t-12} + n_{t-20} + \cdots) + (5/2)(n_{t-10} + n_{t-18} + n_{t-26} + \cdots) - (9/4)(n_{t-16} + n_{t-24} + n_{t-32} + \cdots). \quad (15)$$

This is the second exact formula, Eq. 9 having been the first. By this second formula, a table can be built up with every other year missing, as will be seen in Table 2.

By similar methods it might be possible to find a formula showing n_t as a function of only $n_{t-4}, n_{t-8}, n_{t-12}, \ldots$. The authors, however, have not been successful in this, nor in the limiting ideal solution which would show n_t rigorously as a function only of the number living any desired number of years previous to t. The simple approximation (Eqs. 19 and 20) later to be derived, will, however, give very good results beyond the range of Table 2.

It should be mentioned that in using the formulas, any n occurring with a negative suffix is to be counted as nil; e.g. in applying Eq. 9 at $t=11$ we should have (see Table 1) $n_{t-1}=1434.375$, $n_{t-2}=1106.25$, $n_{t-10}=100$, $n_{t-8}=200$, $n_{t-9}=150$, the values of $n_{t-16}, n_{t-18}, \ldots$ being counted zero, on account of the negative suffixes. Substitution in Eq. 9 gives

$$n_{11} = 1434.375 + \tfrac{1}{2}(1106.25 + 100 + 0) - (3/2)(200 + 0) + (150 + 0) = 1887.5$$

as shown.

Every entry in Table 1 can be found from preceding entries by using Eq. 9 or 15 in this manner.

4. An approximation for large values of t. Eq. 9 can be written like this:

$$\frac{n_t}{n_{t-1}} = 1 + \frac{1}{2}\frac{n_{t-2}}{n_{t-1}} + \left[\frac{n_{t-10}}{2n_{t-1}} + \frac{n_{t-9}}{n_{t-1}} - \frac{3n_{t-8}}{2n_{t-1}}\right] + \left[\frac{n_{t-18}}{2n_{t-1}} + \frac{n_{t-17}}{n_{t-7}} - \frac{3n_{t-16}}{2n_{t-1}}\right] + \left[\frac{n_{t-26}}{2n_{t-1}} + \frac{n_{t-25}}{n_{t-1}} - \frac{3n_{t-24}}{2n_{t-1}}\right] + \cdots. \quad (16)$$

Now as t increases, n_t/n_{t-1} will approach a limiting value. This fact is suggested but not proved by the entries in the last column of Table 1, where the ratio n_t/n_{t-1} appears to have pretty well settled down by the time $t=20$. Let

$$x = \text{Lim } n_t/n_{t-1}. \quad (17)$$

Then Lim $n_t/n_{t-2} = x^2$, Lim $n_t/n_{t-3} = x^3$, etc. In the limit, therefore, Eq. 16 leads to the relation

$$\begin{aligned} x &= 1 + 1/2x + x^{-9}[\tfrac{1}{2} + x - 3x^2/2] + x^{-17}[\tfrac{1}{2} + x - 3x^2/2] \\ &\quad + x^{-25}[\tfrac{1}{2} + x - 3x^2/2] + \cdots \\ &= 1 + 1/2x + [\tfrac{1}{2} + x - 3x^2/2][x^{-9} + x^{-17} + x^{-25} + \cdots] \\ &= 1 + 1/2x + [\tfrac{1}{2} + x - 3x^2/2]/(x^9 - x) \end{aligned} \quad (18)$$

which is to be solved for x. Incidentally, this equation has only one positive root. Table 1 furnishes 1.3056 as an approximation to start with; this value replacing x on the right-hand side of Eq. 18 gives

$$x = 1.30565037$$

for a second approximation, which in turn used on the right-hand side of Eq. 18 gives

$$x = 1.305650533$$

for the third approximation, which is final to as many decimals as written. We can then say that

$$\text{and} \qquad \left.\begin{aligned} \operatorname{Lim} n_t/n_{t-1} &= 1.305650533 \\ \operatorname{Lim} n_{t+k}/n_t &= 1.305650533^k \end{aligned}\right\}. \tag{19}$$

It will be seen that the last few entries of n_t/n_{t-1} in Table 1 fluctuate around the limiting value, 1.305650533, suggesting that Eq. 19 may eventually be used for forecasting the population under the stated assumptions. Table 2 was constructed to test such a proposal: the column n_t therein is exact, having been filled in by the use of Eq. 15, whilst the column $x^2 n_{t-2}$ was calculated as an approximation for comparison, x being taken as 1.305650533. Beyond $t=34$ the error in projecting two years at a time is clearly exceedingly small, and is apparently diminishing with rapidity; moreover the signs of the error are sometimes positive, sometimes negative. The cumulated error over the 14 year period between $t=20$ and $t=34$ is 0.00588 percent, or about 6 parts in 100,000. The cumulated error over the 16 year period between $t=34$ and $t=50$ is 0.00005 percent, or only 5 parts in 10 million.

5. The exponential law of growth. The irregularities in the first part of Table 1 are thus seen to subside as time goes on, whereupon eventually the limiting ratios expressed by Eq. 19 will apply with sufficient accuracy. For instance, if one wished to apply the approximation after $t=50$ years, he would write Eq. 19 in the form

$$\log n_t = \log 620425.045 n_{50} + (t - 50) \log 1.305650533, \tag{20}$$

basing his projections on the number of females living at the beginning of the 50th year. The equivalent exponential form would be

$$n_t = n_{50} \cdot 1.305650533^{t-50}. \tag{21}$$

A study of the errors between $t=40$ and 50 in Table 2 leads to the conclusion that these formulas may be used for forecasting the number of females present in any year, in this idealized situation, over a period of 1000 years with an accuracy of 1 part in a million.

In view of this and the discussion in the preceding paragraph regarding the errors in the use of Eq. 19, it seems safe to argue that Eqs. 19–21, along with

the Tables 1 and 2 (or their mathematical equivalent, Eqs. 9 and 15), constitute a satisfactory if not elegant solution to the question proposed.

6. Some variations of the problem. In the problem just considered, each ewe gives birth to a lamb on her second, third, . . . , and seventh birthdays, and is discarded before her eighth. If each ewe is discarded a year earlier, so that the lamb born on her sixth birthday is the last, the equations are all changed. The limiting ratio n_t/n_{t-1} is now the only positive root of

$$x = 1 + 1/2x + (\tfrac{1}{2} + x - 3x^2/2)/(x^8 - x), \tag{22}$$

this root being $x = 1.2760099$. Eq. 22 differs from Eq. 18 only in having x^8 in place of x^9 in the last parenthesis. By varying the year of discard, we get other values for n_t/n_{t-1} according to the following table.

TABLE 3

Lambs born on each ewe's	Limiting ratio n_t/n_{t-1}
2d birthday only	$1/\sqrt{2} = 0.707107$
2d and 3d birthdays	1
2d, 3d, and 4th birthdays	1.147109
2d,. . . , 5th birthdays	1.228190
2d,. . . , 6th birthdays	1.276100
2d,. . . , 7th birthdays	1.305651
2d,. . . , 8th birthdays	1.324718
2d,. . . , 9th birthdays	1.337335
.	.
.	.
.	.
2d, 3d, 4th, and every one thereafter, the life span being infinite	1.366026

The state of affairs corresponding to the top row of Table 3 is that the flock is not maintaining itself, and the population is actually decreasing; the number living one year is only $1/\sqrt{2}$ of the number living one year previously. The situation in the second row of Table 3 is the flock exactly maintaining itself, each ewe in her life of four years producing one female and one male. In all the other entries in Table 3, the flock is gaining from one year to the next. In the bottom row, each ewe lives forever and produces a lamb every year beginning at the second; in any year there will then be 1.36 ($=\frac{1}{2}+\frac{1}{2}\sqrt{3}$, to be exact) times as many females in the flock as there were the year before. This comes by solving

$$x = 1 + 1/2x, \tag{23}$$

the last term of Eq. 22 having disappeared because of the presence of $x > 1$ raised to an infinite power. The limiting ratio 1.36 for the infinite life span is curiously only 3 percent higher than the limiting ratio that exists when each ewe is discarded at her 9th birthday.

7. Relation to previous work. The novelty in the present work is the fact that it deals with the discrete numbers in the flock during the initial years of growth, the fluctuations in the rate of increase manifested by n_t/n_{t-1} in Table 1 gradually fading out as the growth settles down to the inevitable exponential curve (Eq. 21). After this curve has become a sufficiently good approximation, one may deal with the problem in all its phases by the methods of the infinitesimal

calculus, after the manner of Lotka and others. The attenuation of the oscillations of n_t/n_{t-1} in the present solution bear a striking resemblance to the curve of growth shown by Lotka in a different problem (Lotka, "The spread of generations," *Human Biology* **1**: 305–320, 1929, figures 4, 6, 7, and 8).

SUMMARY

The solution of an ideal problem involving the growth in a flock of sheep is presented with the thought that it might be of use to those having similar problems involving the growth of livestock populations or other growing colonies.

Using the assumptions of this ideal problem, namely, that one starts with any number of newborn female lambs at the commencement of year 0, and that each female gives birth to a lamb when she is two, three, four, five, six, and seven years old, half the lambs being female, and all sheep to be discarded at the end of their seventh year, exact formulas are derived for obtaining the number of females in the flock at any given year when the number of females present either the year previous, or two years previous, is known. It is also demonstrated that the increase of the population finally follows the inevitable exponential law of growth, so that after about fifty years an empirical approximation will give the number of females present in any year thereafter over a period of a thousand years with an accuracy of a few parts in a million. Tables are given to show the growth of the population and the accuracy of the estimates; also solutions for certain variations of the problems.

The analysis treats the growth in its initial stages and follows it into the smooth exponential rate. Competition in food supply and other complications are ignored; these, however, are usually important only later on, wherefore the picture here presented may give a useful approximation to the early stages of growth in a colony.

THE EFFECTS OF THYROID FEEDING ON GROWTH PROCESSES AND RETROGRESSIVE CHANGES IN BONE AND CARTILAGE OF THE IMMATURE GUINEA PIG*

By

MARTIN SILBERBERG AND RUTH SILBERBERG

From the Laboratory of Research Pathology, Oscar Johnson Institute, Washington University School of Medicine, St. Louis

(*Received December 5, 1938*)

A considerable number of data have been collected which to a certain extent provide an understanding of the relations which exist between disturbances of the thyroid gland and pathologic conditions in the skeleton. However, very little is known concerning the effect of experimentally produced hyperthyroidism on growth and ossification of cartilage; furthermore, the histological findings recorded so far (Stefanescu,[19] Askanazy and Rutishauer,[1] Coryn,[6] Martos[14]) are not concordant. We have undertaken, therefore, an experimental study of the rôle played by the thyroid gland in the process of endochondral ossification and of the influence exerted by the thyroid hormone on the cartilage of the joint. The present report deals with the effects of feeding Armour's thyroid tablets to young male and female guinea pigs.

MATERIAL AND METHODS

The bones which served for these studies were obtained from experiments which Dr. Leo Loeb conducted for other purposes and the results of which will be published at a later date. We are greatly indebted to Dr. Loeb for the permission to make use of this material.

Twenty-two male and female guinea pigs weighing between 180 and 195 grams at the beginning of the experiments were fed Armour's thyroid tablets daily for 2, 10, 15, 20 and 30 days. Eleven animals received a daily dose of 0.2 grains of thyroid tablets and the other eleven 0.6 grains daily.

The animals lost weight during the first ten days of the experiment, but, as a rule, regained their initial weights after twelve to fifteen days. Subsequent to this period the weights increased gradually, and thirty days after the beginning of the thyroid feeding the gain averaged 75%.

At autopsy the method of removing tibia, ribs and parts of the spine reported in our preceding papers was applied; likewise the technique of decalcifying, cutting and staining used by us has already been fully described.[16]

MICROSCOPICAL OBSERVATIONS

1. *Epiphyseal line:* After two days' feeding, the zone of endochondral ossification is of medium width. The cartilaginous cell-layers are sharply demar-

* These investigations were carried out with the aid of grants from The International Cancer Research Foundation and from the Committee on Scientific Research of the American Medical Association.

cated and have a regular and fairly normal structure. After ten days a slight hyperplasia of the resting and columnar cartilage zones occurs: instead of ten cells which are normally found in one cell-row, the average may be as high as twelve. The nuclei of the resting cells are enlarged and take on a dark blue stain with hematoxylin; the lighter cytoplasm increases in size and is oval in shape. Thus, the conversion of the resting into columnar cartilage cells is enhanced. Not infrequently, particularly at about twenty days after the beginning of the thyroid feeding, the columnar cartilage cells are seen to proliferate by way of mitoses, whereas, under normal conditions, these cells usually proliferate by amitosis. The average cell count may now reach twelve to thirteen cells but the histological pattern is rather indistinct.

After fifteen to twenty days the epiphyseal line is somewhat wider than under normal conditions. There are more hypertrophic cartilage cells seen in a single cell-row as compared with the normal condition. The number of such cells in one column which ordinarily averages four, may now be as high as six. Mitoses were not seen in the hypertrophic cartilage.

Under normal conditions one row of cartilage cells consists of about ten columnar and four hypertrophic cells. In the following table the first figure indicates the number of hypertrophic cells and the second figure the number of columnar cells. These figures, therefore, indicate the changes in the number of cells of each kind which take place under the influence of thyroid feeding, and in addition, they show the ratio between the hypertrophic and columnar cells. For instance, after fifteen days of thyroid feeding one finds in female guinea pigs six hypertrophic cells instead of the four, and eleven columnar cells instead of the average ten.

The figures given represent averages of the individual counts. We classify as hypertrophic cartilage cells those whose length is at least as great as their width.

TABLE

AVERAGES OF NUMBERS AND RATIOS OF HYPERTROPHIC AND PROLIFERATING CARTILAGE IN A CELL COLUMN

Period	0.2 grains thyroid substance daily		0.6 grains thyroid substance daily	
	Male	Female	Male	Female
2 days	—	4–5:11	—	5:12
10 days	4–5:12	5–6:11	4:10–11	5:11
15 days	4–5:10–11	6:11	5:12	5:11
20 days	6:11	5:11	5:11–12	5:11–12
30 days	5:13	5:12–13	6:12	5:11

If we now consider the replacement of the cartilage cells by bone, this does not take place at the same pace throughout the area of ossification. In some areas of the epiphyseal layer the formation of bony spiculae can be seen; however, these trabeculae appear thinned out, their outlines showing irregular grooves which are filled with multinucleated giant cells acting as osteoclasts; but in other places strands of calcified, hypertrophic, but not yet ossified or only incompletely ossified cartilage are seen protruding into the marrow instead of

the normal osseous trabeculae with their regular outlines. These strands are surrounded by a more or less well developed osteoblastic tissue with epitheloid polyedric or oval osteoblastic cells, arranged in a bead-like manner and situated in a dense lymphoid marrow. These changes are most pronounced after fifteen to twenty days and are seen in both sexes. After that time they gradually recede.

After a ten to fifteen days' treatment the intercartilaginous matrix, which is present in about the usual quantity, takes on a blue stain, a little darker than normal, with hematoxylin. In addition it may undergo a slight swelling and vacuolization which, as stated above, make the outlines of the cells somewhat indistinct. This is the condition under which the mitotic division of the cartilage cells may be readily observed. Still more marked retrogressive alterations are lacking.

2. *Chondrophyte:* The changes which are found in the triangular lateral protuberances correspond to those seen within the epiphyseal line; in particular, one notes an increased conversion of precartilaginous cells into cartilage cells. The cartilage cells adjoining the zone of endochondral ossification are hypertrophic. With an increasing dose of thyroid and a longer duration of the experiment, the changes become more marked, and in advanced cases either a fusion or a degeneration of such hypertrophic cartilage cells may be seen. The proliferative and hypertrophic processes, however, are not as pronounced as after administration of anterior pituitary substance, but are comparable rather to those which we have observed after castration of young male guinea pigs.[17]

3. *Joints:* The earliest alterations are seen in the innermost zone of cartilage which in the beginning is considerably wider than under normal conditions, owing to an increase in the number of hypertrophic cartilage cells. Loops of capillaries growing out of the bone marrow advance toward this cartilaginous cell-layer. They cause a corrosion, thinning, and finally a complete absorption of the bony border lamellae. At later stages they penetrate into the zone of the hypertrophic cartilage cells which, by means of numerous multinucleated chondroclasts developing in the meantime, are readily opened and broken down. Still later, the capillary loops corrode and perforate the pressure zone of the joint cartilage and invade also the zone of the transitional cartilage. Thus, the cartilaginous covering of the joints is gradually reduced in thickness. Finally, minute arthropathic lesions may result which are not unlike those we have observed subsequent to castration,[17] although they may be of lesser degree. Simultaneously with these lesions the cells of the transitional zone proliferate slightly and become perpendicular in arrangement. They increase in size and take on a darker blue stain with hematoxylin; the stroma is accordingly diminished.

4. *Bone marrow:* The vessels of the lymphoid marrow are enlarged and congested in the epiphysis as well as in the diaphysis, in the proximal part of the marrow as well as in the distal. In the subepiphyseal layer and extending from the periphery towards the center, a polymorphous, well vascularized, loose connective tissue develops, which gradually replaces the normally differentiated lymphoid marrow. It is rich in cells, showing numerous mitoses, and it accompanies the above mentioned strands of incompletely ossified hypertrophic car-

tilage. The changes are the more pronounced, the greater the dose of thyroid substance and the longer the period of treatment. Thus after twenty to thirty days, instead of the normal lymphoid marrow, areas of a fibrous tissue containing many multinucleated osteoclasts may be found which cause an absorption of the bony trabeculae and of the bony cortex. Here and there, extravasates of blood are seen within the fibrous tissue. The larger the amount of newly formed vascularized granulation tissue, the more readily absorption of the bony substance occurs. The marrow in ribs and vertebrae undergoes corresponding changes.

5. *The bony shaft:* The periosteum is widened and consists of a vascular loose connective tissue with numerous spindle cells which multiply rapidly by way of mitotic cell division. These mitoses are found in great numbers along the bony shaft. At later stages the mass of this parostal tissue increases more and more, and simultaneously multinucleated giant cells are formed which act as osteoclasts. They are situated in excavations of the compacta and help to absorb and finally to destroy the bony substance. Not infrequently they form ribbons and are separated from each other by thin osseous spurs, remnants of the original bony substance. Thus, the surface of the bone becomes rough and depressions occur due to the formation of fairly large lacunae; this condition, together with the ingrowth of capillary buds from the marrow and endosteum, finally results in a greater and greater rarefication of the shaft. Furthermore, minute infractions may occur, the spaces between the fractured parts being filled with a richly cellular, actively proliferating connective tissue. Osteoblasts are also present and line the endosteum, but the resorptive processes predominate over the apposition of bone by osteoblasts.

6. *Ribs:* The reactions of the cartilage and bone of the ribs correspond to those seen in the long bones. Ten days after the beginning of the experiment a slight degeneration takes place in some of the resting cartilage cells; their nuclei become pycnotic and the cytoplasm enlarges. The chondromucoid ground substance is somewhat swollen and vacuolated, a condition similar to that seen in the epiphyseal line of the long bones. Due to increased absorption, after twenty to thirty days minute infractions occur at the chondro-osseous junction, with granulation tissue filling the spaces between the bony remnants. This tissue is fairly abundant and represents the substratum for the rosary-like swelling seen grossly at the chondro-osseous junction.

7. *Vertebrae:* Here the conditions are similar to those observed in other bones. There is an increased absorption of the bony substance and an increased tendency toward fibrosis of the marrow, but no accelerated ossification.

DISCUSSION

Administration of thyroid substance over periods of from two to thirty days exerts a stimulating effect on growth and differentiation of the euhyaline cartilage of epiphyses and joints. It induces a slight proliferation of the resting cartilage, accelerates its development into columnar cartilage, and the conversion of the latter into hypertrophic cartilage. Associated with these processes is the

production of a strongly proliferating vascular connective tissue within the bone-marrow and the periosteum. This causes a breakdown of the bony trabeculae and of the compacta of the bony shaft. In advanced cases the fibrosis of the bone-marrow is not unlike that seen in the case of experimental hyperparathyroidism, although it is less in degree (Jaffe et al.[11]). The various changes are not limited to the long bones but occur also in ribs and vertebrae, independently of the sex of the animals.

In order to evaluate the findings in the various constituents of the epiphyseal lines, joints and bony shafts, it may be advisable to consider the results obtained in these areas separately.

1. *Resting and columnar cartilage:* Although hyperplasia of these cells from active stimulation of thyroid substance is present, it is slight as compared with the increase in the number of these cells after administration of acid extract of anterior pituitary gland, and it is less marked even than that found after gonadectomy. The response of the male is apparently more retarded than that of the female. It is possible that the slight hyperplasia which is observed at these early stages may be due to the increased metabolic rate induced in the organism by the thyroid feeding as a result of which larger amounts of nutritional material are made available to the cells.

As to the numerous mitoses which appear here in the columnar cartilage, these were not found following administration of anterior pituitary substance nor after gonadectomy. They may be due perhaps to a specific effect of the thyroid hormone which, in certain concentrations, is able to raise the mitotic index in tissue cultures (Carrel,[4] Ebeling[8]) as well as in cultures of paramecia (Shumway,[15] Cori,[5] Budington and Harvey[3]). However, such a stimulating effect does not seem to apply universally, inasmuch as Woodruff and Swingle[22] could not detect it in paramecia. In the living mammalian organism Loeb[13] and coworkers found that thyroid feeding does not elevate the number of mitoses in the epidermis of guinea pigs nor does it cause hypertrophy. Moreover, other investigators have found thyroxin to cause a depression of the mitotic index in tissue culture of fibroblasts,[12] and in cultures of paramecia,[22,21] in phallusia and echinometra,[20] rather than an elevation.

2. *Hypertrophic cartilage:* The number of the hypertrophic cartilage cells is relatively more increased than that of the resting and columnar cartilage cells. This may be due to an increased or accelerated conversion of columnar into hypertrophic cartilage cells or to a retarded transformation of the latter into bone, or it may be due to both of these processes. Since it has been shown by Gudernatsch[9] for amphibian larvae, by Dott[7] for mammals, and by Budington[2] for plants that thyroid hormone may accelerate the process of differentiation of tissues, and since hypertrophy of the growing cartilage cells may be considered as a process related to differentiation, the latter change may likewise be due to the direct action of thyroid hormone. In this connection we may refer also to the observation of Hammett[10] on the effects of thyroid gland on the growth of the rat; he concluded that growth induced by thyroid activity consists in an increase in cell mass rather than in cell number.

In addition, however, the possibility of a retarded ossification cannot be excluded. It must be noted that although chondroclasis is accentuated particularly in the cartilage of the joints, in the bone-marrow long strands of hypertrophic cartilage are seen which have not or have only incompletely been ossified. These strands are identical with those which we have observed in male guinea pigs subsequent to castration.[17] The results of the experiments reported in this paper support the assumption that the thyroid hormone is responsible for the production of these structures. The retardation of ossification may be due, at least partly, to the fact that, to judge from the histological appearances, the intercartilaginous matrix surrounding the hypertrophic cartilage cells contains amounts of calcium salts which seem to be increased as compared with the normal. This finding is in agreement also with the recent observation of Smith and McLean,[18] who showed by means of chemical analysis that after administration of thyroid substance the calcium content of the long bones is not decreased despite the marked osteoporosis of the bony shafts which has been noted by the various investigators and which we also have observed. It is probably the abundant deposition of calcium in the hypertrophic cartilage which, in the final balance, makes up for the loss of calcium due to the absorption of bone. We may assume that in order to become ossified this hypercalcified cartilage requires increased activity of osteoblastic tissue. Furthermore, it seems probable that the strong tendency to the absorption of bone by means of osteoclasis inhibits this osteoblastic activity.

SUMMARY

In young guinea pigs, administration of thyroid hormone up to thirty days causes a slight hyperplasia which may take place by mitosis, a marked hypertrophy and an accelerated differentiation of the euhyaline cartilage of the epiphyseal line, ribs, joints and vertebrae. The subsequent replacement of the hypertrophic cartilage by bone is retarded. Increased absorptive processes, due to the activity of bone-marrow cells and of periostal cells, lead to a porosis of the compact bone as well as to an arthropathic rarefication of the cartilaginous covering of the joints. The changes are most pronounced after twenty days. The response of the male to the action of thyroid feeding is somewhat retarded as compared with that of the female animal.

REFERENCES

1. Askanazy, M. and E. Rutishauer. 1933. *Virchows Archiv fuer Patholog. Anat.* **291**: 653–681.
2. Budington, R. A. 1925. A Suggestion as to the Effect of Thyroid Gland Substances on Protoplasm in General. *Biol. Bull.* **48**: 83-91.
3. Budington, R. A. and H. F. Harvey. 1916. Division Rate in Ciliate Protozoa as Influenced by Thyroid Constituents. *Biol. Bull.* **28**: 304–314.
4. Carrel, A. *Cit.* Ebeling, A. H. #8.
5. Cori, G. T. 1923. The Influence of Thyroid Extract and Thyroxin on the Rate and Multiplication of Paramecia. *Am. Jour. Physiol.* **65**: 295–299.
6. Coryn, G. 1938. Les Affections Endocriniennes du Squelette. *Presse Méd.* **46**: 228–231.
7. Dott, N. M. 1923. An Investigation into the Function of the Pituitary and Thyroid Glands. *Quart. Jour. Exper. Physiol.* **13**: 241–282.

8. EBELING, A. H. 1924. Action de Epithélium Thyroïdien in Culture Pure sur la Croissance des Fibroblasts. *Compt. rend. Soc. Biol.* **90:** 1449–1450.
9. GUDERNATSCH, J. F. 1914. Feeding Experiments on Tadpoles. *Am. Jour. Anat.* **15:** 431–473.
10. HAMMETT, F. S. 1926. Studies of the Thyroid Apparatus. *Am. Jour. Physiol.* **77:** 527–547.
11. JAFFE, H. L., A. BODANSKY, and J. E. BLAIR. 1931. Fibrous Osteodystrophy (Osteitis Fibrosa) in Experimental Hyperparathyroidism of Guinea Pigs. *Arch Path.* **11:** 207–228.
12. LATTA, J. ST. and J. Z. DAVIS. 1938. The Effects of Thyroxin in Varying Concentrations on the Growth of Fibroblasts in Vitro. *Arch. f. Exper. Zellforsch.* **21:** 427–435.
13. LOEB, L., F. L. HAVEN, I. T. GENTHER, and H. FRIEDMAN. 1930. The Effect of Administration of Thyroid Substance, of Potassium Iodide, and of Extirpation of the Greater Portion of the Thyroid Gland on the Epidermis of the Guinea Pig. *Anat. Record.* **46:** 65–80.
14. MARTOS, J. 1938. Knochenveraenderungen bei experimentellem Hyperthyroidismus und bei Basedow-Krankheit. *Beitr. pathol. Anat.* **100:** 293–308.
15. SHUMWAY, W. 1917. The Effects of Thyroid Diet upon Paramaecium. *Jour. Exp. Zool.* **22:** 529–563.
16. SILBERBERG, M. and R. SILBERBERG. 1938. Effects of Anterior Pituitary Implants and Extracts on Epiphyses and Joints of Immature Female Guinea Pigs. *Arch. Path.* **26:** (1208–1225).
17. SILBERBERG, M. and R. SILBERBERG. 1939. Growth Processes in Cartilage and Bone Subsequent to Gonadectomy and Administration of Anterior Pituitary Extract of Cattle in Immature Male and Female Guinea Pigs. *Am. Jour. of Path.* **15:** (in press).
18. SMITH, E. E. and F. C. MCLEAN. 1938. Effect of Hyperthyroidism upon Growth and Chemical Composition of Bone. *Endocrinology* **23:** 546–552.
19. STEFANESCU, M. 1926. Les Modifications des cartilages de conjugaison chez les animaux hyperthyroïdés. *Compt. rend. Soc. Biol.* **95:** 1570–1572.
20. TORREY, H. B. 1928. Thyroxin as a Depressant of Cell Division; its Effect on the Cleavage and Early Development of Sea Urchin and Ascidian. *Endocrinology* **12:** 65–80.
21. TORREY, H. B., M. C. RIDDLE, and J. L. BRODIE. 1925. Thyroxin as a Depressant of the Division Rate of Paramecium. *Jour. Gen. Physiol.* **7:** 449–460.
22. WOODRUFF, L. L. and W. W. SWINGLE. 1924. The Effects of Thyroid and Some Other Endocrine Products on Paramecium. *Am. Jour. Physiol.* **69:** 21–34.

A COMPARISON OF CERTAIN GLAND, ORGAN AND BODY WEIGHTS IN SOME AFRICAN UNGULATES AND THE AFRICAN ELEPHANT

By

D. P. QUIRING

Western Reserve University, and Cleveland Clinic Foundation

(*Received December 7, 1938*)

In the winter of 1935–36 members of the Cleveland Clinic expedition to South-East Africa made a series of dissections of the following named animals:

Common Name	*Scientific Name*
Buffalo East African	*Syncerus caffer radcliffei*
Masai Bushbuck	*Tragelaphus scriptus massaicus*
Burro	*Equus africanus*
Naivasha Kirk Dik-Dik	*Rhynchotragus kirki cavendishi*
*Elephant (East African)	*Loxodonta africana capensis*
Gazelle (Thomson)	*Gazella thomsoni*
Giraffe	*Camelopardales tippelskirchi*
Hippopotamus	*Hippopotamus amphibius*
Horse	*Equus caballus*
Hyrax	*Heterohyrax brucci*
Impala	*Aepyceros melampus*
Coast Hartebeest	*Bubalis cokei cokei*
Mule	*Equus africanus*
Swahili reedbuck	*Redunca redunca tohi*
Rhinoceros	*Rhinocerus bicornis*
Steinbok (Masailand)	*Raphicerus campestris neumanni*
Warthog	*Phacochoerus aethiopicus*
Waterbuck (Highland)	*Kobus ellipsipyrynnus thikae*
Wildebeest (Athi White-bearded)	*Gorgon albojubatus albojubatus*
Zebra (Highland Quagga)	*Equus quagga granti*

In the course of these dissections, body, brain, thyroid gland, adrenal body, heart, kidney, liver, and lung weights were taken (Table 1). In some instances intestinal weights were secured as well. The weighings were made immediately after the animals had been killed. Small specimens were weighed entire with a 600 pound capacity Chatillon scale; the larger were weighed with this same scale after sectioning into pieces suitable for the capacity of the scale. Glands and organs were weighed with a small Chatillon autopsy scale or with a Cenoco beam balance.† For comparative purposes a few data taken from American perissodactyls have been included.

* Characterized by four hoofs in the front and three in the hind foot in contrast to the West African species which has five and four respectively (Scott, p. 273).

† The weighing of the elephant presented a real problem because of his great weight (6654.78 kg.), and while the number of individual weights which were necessary to secure his total weight introduced a factor of error, extreme care was exercised in order to achieve accuracy.

Because of the close affinities of the various animals represented in this study, an attempt was made to apply the power formula, $Y = bX^k$ to the subordinate gland, and the body weight relationships. This equation has come into common use the past few years and today is generally known as the relative growth formula through the work of Huxley (1932) although as long ago as 1891 Otto Snell applied it in determining what he called the "cephalization coefficient" of the brain of various mammals. DuBois in 1898 took over Snell's concepts and applied the formula to explain the degree of brain development for a

TABLE I

UNGULATA AND SUBUNGULATA*

Name	No.	Sex	Body Weight	Adrenal	Thyroid	Brain	Heart	Kidney	Liver	Lung
Dik-dik	1	M	4.575	.56	.73	37.	36.80	21.40	93.22	44.36
Steinbok	2	M	8.62	1.35	1.22	49.52	72.22	38.11	176.15	151.90
Reedbuck	2	M	31.81	2.11	1.34	108.7	242.	102.	559.	431.
T. gazelle	2	M	24.43	2.00	1.83	91.8	245.	106.	528.	281.
Impala	2	M	37.86	5.96	5.67	148.5	270.	161.	606.	510.
Impala	2	M	57.27	4.72	5.04	175.	378.	225.	747.	730.
Bushbuck	1	M	53.06	5.09	5.08	190.	350.	225.	1100.	540.
Warthog	1	M	65.45	8.24	3.60	125.	325.	300.	1500.	550.
Hartebeest	1	M	134.09	9.61	1.83	275.	875.	387.	1525.	1850.
Waterbuck	1	M	188.20	6.1	9.57	290.	1075.	410.	2135.	1975.
Wildebeest	2	M	212.23	8.42	7.36	443.	1312.	497.	2278.	2850.
Zebra	2	M	254.72	23.08	18.21	541.	1985.	838.	4017.	2025.
Zebra	1	F	297.05	27.80	17.43	555.	1970.	900.	4400.	2790.
Buffalo	1	F	571.88	43.27	37.30	642.	3050.	1625.	7175.	5150.
Buffalo	2	M	760.90	37.50	35.99	653.	3620.	1825.	7709.	8110.
Rhino	1	M	765.45	88.00	53.05	655.	4800.	3000.	14310	7350.
Giraffe	1	M	1198.00	78.12	64.70	700.	4990.	2260.	19050	12060
Hippo	1	F	1350.48	61.05	118.71	720.	4535.	3160.	23.580	11.339
Elephant	1	M	6654.78	940.0	860.0	5712	26077	18140	10.667	138.790
Hyrax	1	M	.750	.161	.081	12.	3.	6.45	3.153	5.532
Thorobred mare	1	F	546.27	60.78	26.45	652.	4435	2210	6903	4085
Panama burro	2	M & F	121	8.5	4.0	303.				
Shet. pony	1	M	150.33	16.29	14.80	496.	1375			
Panama mule	3	M	211.03	24.76	8.66	388.				
Panama mule	4	F	290.86	35.50	9.75	500.				
Panama horse	8	M	278.87	25.75	10.77	451.				
Panama horse	31	F	261.75	25.62	12.77	467.				

* Body weights given in kilograms; others in grams.

wider range of mammals than did Snell. In the same year, 1898, Lapicque found that the equation could be applied to brain-body weight relations of birds. More recently the equation has been applied to a great variety of relationships of subordinate parts to bodies. Hersh (1934) has applied it to skull relationships in various genera of titanotheres.

As the equation is commonly used Y represents the subordinate part; b is the value of Y when X is made equal to 1, while X stands for the body weight or the independent variable. k represents the slope of curve when Y is plotted against X on a double logarithmic grid. Snell interpreted the constant b in a novel way. He had found that the k value was fairly constant from species to species. b on the other hand varied with the species. In a group of animals of approximately the same degree of brain development, b values tended to be

equal; such animals Snell and DuBois called iso-neural. Increase in b represented increase in the degree of neural development according to these workers. On this basis Snell and later DuBois called b the "cephalization coefficient" since it gave a numerical expression for the degree of cephalization. Graphically it would be indicated as the y intercept of the curve. It becomes obvious that it changes from species to species and in comparisons between classes, differences in b become quite large, e.g., in human beings the b for brain is 2.92, in the mouse 0.1025.

BRAIN

The brains in the compared animals ranged in weight from 12.27 grams for the hyrax to 5,712 grams for the elephant. When plotted on double logarithmic grids (Fig. 1) against the body weights the values fall into a narrow band with a slope of approximately .5410–.5703, which represents the k value of our equation. This value was arrived at by employing the equation $k = \frac{\log y_1 - \log y}{\log x_1 - \log x}$ and using the values for the first ten animal groups against the next ten (Table II). Such individual comparisons, while they show wide deflections from a value of .56, particularly in the case of the giraffe and the elephant, nevertheless indicate the trend.

In view of the great variety and the wide weight range of the compared forms this rather close approximation to the values found by earlier workers appears

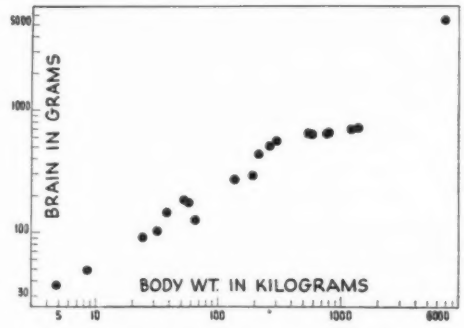


FIG. 1. Brain-body weight relationships of the compared groups, showing the high relative value for the elephant brain (last point on the graph).

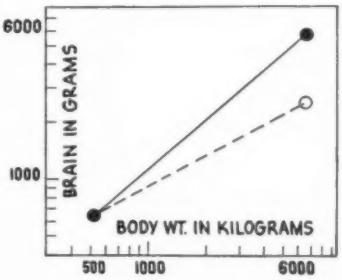


FIG. 2. A comparison of the brain weight of the elephant and the thoroughbred mare. The interrupted line shows the theoretical weight of the elephant brain if it followed the ungulate pattern, the solid line shows the real relationship.

to point to the correctness of their conclusions with respect to k, namely, that it approaches a constant value for the brain-body weight curves regardless of the class of animals on which it is employed and that differences appear in b rather than in k.

It will be noted that where the elephant data are employed in table II the k values are high; in addition the locus for the point for the elephant brain is considerably above that for the group as a whole. If the elephant brain weight followed that of the larger ungulates the brain weight would be only about one-

TABLE II
UNGULATA
k values

Animals compared	Brain	Heart	Adrenal	Thyroid	Liver	Kidney	Lung
Dik-dik vs waterbuck	.553	.904	.639	.698	.844	.790	1.015
Steinbok vs wildebeest	.574	.902	.627	.674	.792	.799	.922
Thomson gazelle vs zebra	.752	.974	1.043	.976	.866	.910	.721
Impala vs zebra	.638	.964	.589	.798	.964	.847	.822
Impala vs buffalo	.405	.912	.968	.878	.928	.863	.924
Hartebeest vs buffalo	.427	.822	.891	1.065	.934	1.026	.986
Reedbuck vs rhinoceros	.564	.938	1.171	1.146	1.019	1.057	.884
Bushbuck vs giraffe	.377	.504	.874	.826	.916	.749	1.002
Warthog vs hippo	.578	.869	.661	1.157	.862	.779	.999
Mare vs elephant	.835	.712	1.095	.9712	1.087	.842	1.401
Averages	.541	.850	.855	.893	.921	.866	.967

half that of its actual weight. This theoretical weight of the elephant brain may be determined from the equation derived from the proportion

$$Y:Y_1::X^k:X_1{}^k \qquad \text{or} \qquad Y = Y_1\left(\frac{X}{X_1}\right)^k.$$

Using the impala brain and body weight y_1 and x_1 respectively (average of 4), as a basis for comparison, the equation reads:

$$\text{Elephant brain weights} = 161.65\left(\frac{6654.78}{47.79}\right)^{.541} = 2309.6 \text{ grams.}$$

The relatively great size of the elephant brain is further disclosed (Fig. 2) when compared with the brain weight of the thoroughbred mare. If he followed the ungulate pattern in the matter of brain size the value would fall on the interrupted line with a .541 slope; instead his actual brain weight is just over twice the theoretical weight.

The buffalo brain measured in these terms shows a smaller actual brain weight compared to the theoretical value, namely, 673.2 grams against an actual brain weight of 575 grams. The giraffe brain on this basis weighs 924.7 grams instead of 700 grams. In other words, these two animals appear undersupplied with brains as compared with some of their smaller ungulate relatives.

These divergences in the brain-body weight relationships are brought out strikingly when even-toed ungulates are compared with the odd-toed ones. In the comparison shown in figure 3 we have included data from our domestic horse, mule, and burro. The interrupted line represents the .541 slope followed by the Equidae generally while the large artiodactyls follow a much gentler slope. It is obvious that the large even-toed animals including thoroughbred

horse, mule, and burro, tend toward relatively heavier brains than does the work horse and the rhino. Artificial selection has produced the large work horse, while the rhino has acquired great size naturally, but with relatively lesser brain increase. He is low in brain size compared to the remaining perissodactyls. The hippopotamus, in spite of the fact that he is taking to the water, has a small brain. Commonly we associate a relatively large brain with aquatic mammals. It may be that because of specific brain mutations he has been forced to seek the protection of the water and swamp in order to persist as a species. The hippo's weight is about one-fifth that of the elephant and his brain is relatively smaller than is the elephant brain. If he followed the pattern of the lesser ungulates the hippopotamus brain should weigh 986.70 grams. His actual brain weight is 720 grams or 266.70 grams under the expected value while the elephant brain runs 3402.4 over the expected value.

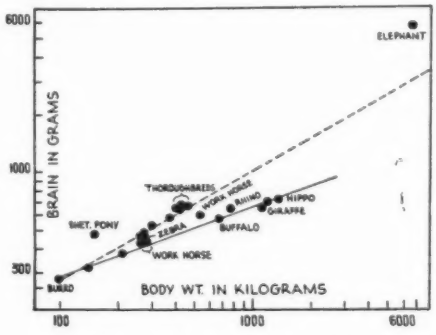


FIG. 3. Broken line shows brain-body weight relations in perissodactyls as compared with large artiodactyls (solid line) and elephant (highest point on graph), slope .541 perissodactyls, slope .33 large artiodactyls.

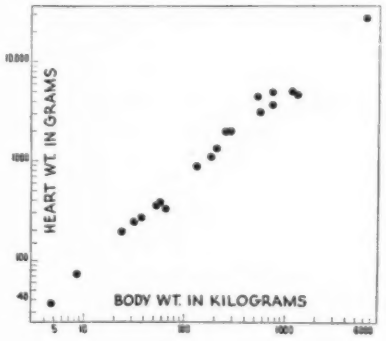


FIG. 4. Heart-body weight relations showing regularity of size increase slope .82.

At first glance these differences in brain size appear to have no effect upon survival value since the hippo, rhino, giraffe, and elephant left to themselves, appear to be in no danger of extinction. Considering the great bulk of the elephant, however, and his slow rate of reproduction plus the problems which arise in connection with the maintenance of his great body, his great heat production, which according to Benedict is about 2000 calories per square meter of surface, we may well infer that because of the acquisition of this great brain he has been able to survive in the face of these very real odds.

The gland weight relationships in the elephant recall the statement of Scott, p. 267 that "the Proboscidea are very isolated among hoofed animals and display in their structure a curious mixture of great specialization in teeth and skull, with extreme conservatism in body and limbs." Their internal organs judging from our specimen appear to reflect these conditions directly as well as the problems involved in maintaining their great bulk.

The modern race horse appears to have had a relatively large brain bred into it among other acquisitions. Measured on a .541 slope against the impala 161.65 $(546.27/47.49)^{.541} = 603.9$ grams, its theoretical weight against 652.9 grams the actual weight.

The b values or cephalization coefficients show a mean value of $.3730 \pm .0109$ and the question arises whether the deflections from the mean simply represent deviations due to the fewness of our samples or whether they indicate real differences in degree of cephalization. It is likely that both factors are involved for fluctuations in b in the two zebra and the two impala groups indicate that marked deviations are present in individual comparisons, on the other hand, the high value of the elephant, bushbuck, wildebeest and zebra and in comparison the value of 2.92 which has been established for the human being, indicate that b does give a clue to degree of cephalization. In comparison with the mean of the Group, Table IV, the buffalo, giraffe, hippopotamus, and warthog appear to lie on a lower cephalization scale than do the others.

HEART

In 1919 Berthold Klatt applied the power equation $Y = b^k$ to heart-body-weight relationships, after the earlier method of Snell and DuBois. In his paper he included some data on birds and on man as well as on certain carnivores. His calculations indicated a value of .8430 for the k value of 22 dogs, .8181 for 433 human males and .7247 for 368 human females. In the human group the body weights ranged from 32.5 to 62.5 kg. for the males and 27.5 to 57.5 kg. for the females. To establish the k values he compared small with large individuals within the species or of closely related species, e.g., dog against fox. In the case of man he compared large males against small males and large females against small females.

The fact that the k value tends to approach 1 indicates the close relation which the heart bears to the body mass. If the k value were .67 it would imply a direct correlation to the body surface since the body weight raised to the .67 power times a constant is equivalent to the surface area. The value .85 stands about half-way between the values .67 and 1. Reference to figure 4 shows that the heart weight increase is very regular in passing from the small to the large animals, indicating the great influence of body mass on the size-relationships of this organ. We have made no attempt to evaluate the efficiency of the various hearts, but we assume that because of the regularity of heart size increase this would be about the same throughout the group. According to Brody "a work horse in good condition is capable of spending about 100 times as much energy for a few seconds as he does when he is absolutely quiet, under conditions of 'basal metabolism'." From the physical condition and appearance of these hearts, I believe that corresponding work output is possible for these wild ungulates.

The heart b values derived from the equation $b = Y/X^k$ which we have designated as cardiac coefficients give a mean value of $0.3509 \pm .000917$. Some interesting differences appear, the most striking being the high b value of the horse

heart and the relatively high position which the elephant holds. This b value for nine thoroughbred horse hearts* is .05821 and indicates the effect of breeding on the cardiac coefficient.† The cardiac b values of the perissodactyls are .0491 as against .0332 for the artiodactyls. Indicated graphically a pair of curves with identical slopes .85 would result with the odd toed group values originating .0491–.0332 points higher along the y intercept than the artiodactyls. Taken individually, a family of lines with identical slopes could be plotted with the thoroughbred horse values at the highest level and warthog, dik-dik, and hippopotamus at the lowest.

We have here then, if the cardiac coefficients represent more than chance deflections, a basis for the evaluation of the heart which is more accurate than the usual heart to body weight ratio since it takes into account the normal rate of heart size increase to body weight increase (by the use of k and b) and we find such factors as systematic position of the animal, degree of tissue vascularization, energy output of heart grouped in the constant b. It becomes obvious that factors enter into the elephant, rhino, zebra, and horse, heart-body weight relationships which are absent in other animals and it appears certain that specific size relationships have been bred into the thoroughbred horse which may be forecast but which are not actually present in the heart of the zebra.

ADRENAL GLANDS

The adrenal glands range in weight from .161 in the hyrax to 940 grams in the elephant. Medullary-cortical relationships likewise vary within rather wide limits. In this study we have confined ourselves to the gross weight relationships, although incidental histological examinations of the glands disclose marked differences, indicated generally by larger medullae in the wild animals as compared with smaller in the domestic.

In passing from the small to the large ungulates a regular size relationship is apparent between the adrenal gland and the body. When plotted against body weights the slope of the curve (double logarithmic) is .866 (Fig. 5). Certain animals fall under the values expected if this relationship holds. Among these are: waterbuck, kongoni, wildebeest, and hippopotamus. On the other side of the curve with adrenal glands heavier than the expected values are: warthog, zebra, rhino, and elephant. In the case of the elephant, the correlation between adrenal gland weight and body weight is almost direct, i.e., the slope has a value of .998. We conclude that just as in the heart, the adrenal gland size tends to approach a direct correlation with the body mass.

The mean adrenal b value$\times 1000$ is 0.4453 ± 0.00358. The adrenal b values (Table III) fall into four general groups with waterbuck, reedbuck, wildebeest in the lowest category; hippopotamus, gazelle, buffalo, kongoni, dik-dik, bushbuck, impala in the second; zebra, thoroughbred mare, giraffe, warthog, steinbok in the third; and rhino and the elephant in the highest group with the elephant heading the list once more. On this basis the elephant possesses the

* Not included in these data.

† Herrmann's data on 16 race horses which show an average ratio of 92.5 grams per kilogram of body weight compare favorably with our own.

highest adrenal coefficient followed by the belligerent rhino, with the zebra and the mare third. It is significant in this connection that all the perissodactyls are in the upper two categories so far as adrenalization is concerned.

TABLE III

b VALUES

Name	Adrenal, *k* at .866	Thyroid, *k* at .89	Brain, *k* at .541	Heart, *k* at .85	Kidney, *k* at .866	Liver, *k* at .921	Lung, *k* at .967
Dik-dik	.000386	.0004116	.3342	.0232	.01858	.03958	.01309
Steinbok	.000519	.0003998	.4518	.0326	.01501	.04179	.02315
Reedbuck	.000264	.0001327	.2841	.0342	.01292	.03981	.01907
T. gazelle	.000317	.0002272	.3203	.0288	.01680	.04800	.02441
Impala	.000412	.0004732	.4061	.0346	.01746	.03680	.01907
Impala	.000376	.0003251	.3582	.0339	.01693	.03603	.01816
Bushbuck	.000412	.0003168	.4284	.0329	.01821	.04873	.01446
Warthog	.000556	.0001862	.2511	.0269	.02025	.05501	.01208
Hartebeest	.000346	.0000497	.3685	.0381	.01396	.02874	.02024
Waterbuck	.000165	.0001934	.3237	.0353	.01109	.02965	.01565
Wildebeest	.000205	.0001984	.4611	.0389	.01212	.02828	.02011
Zebra	.000502	.0004453	.5282	.0489	.01925	.04217	.01299
Zebra	.000506	.0003909	.4783	.0449	.01639	.04008	.01400
Buffalo	.000322	.0001762	.2924	.0387	.01472	.02951	.01669
Buffalo	.000447	.0002803	.3835	.0389	.01678	.03576	.01638
Rhino	.000706	.0003076	.3498	.0479	.02407	.05452	.01501
Giraffe	.000525	.0002517	.2761	.0340	.01231	.04816	.01957
Hippo	.000299	.0003399	.2657	.0284	.01555	.04601	.01339
Elephant	.001157	.0007277	.8626	.0394	.01651	.06806	.03510
Hyrax	.000521	.0002236	.3006	.1306	.02097	.07192	.00917
Mare	.000519	.0002070	.3998	.0588	.02375	.03587	.01157
Shetland pony		.0003628		.0546	.01517	.03057	.04052
Mean	.0004453	.0003194	.3730	.03509	.01655	.03964	.01772

While these differences in *b* may represent deviations from a constant ungulate value they appear to have a deeper significance and may well represent phylogenetic differences expressing themselves in differences in energy output.

To what extent they are reflected in differences in activity pattern is difficult to state. It would appear that the relatively great size of rhino and elephant, as well as the peculiar characteristics of the thoroughbred horse and zebra make new demands upon the adrenal gland in connection with setting these great bodies into rapid movement and for the maintenance of muscle, heart, and vessel tonus. This explanation appears more plausible when the *b* values of the heart are compared with those of the adrenal gland. Thus this same group which heads the list so far as adrenal *b* values are concerned also stands near the head of the list with reference to the heart *b* values. The following table shows this relationship with reference to adrenal and heart mean.

	Adrenal b	Heart b
×1000		
Mean	.4453± .0035	Mean 0.03509±0.000917
	Zebra .503	.0469
	Mare .519	.0588
	Giraffe .525	.0340
	Rhino .706	.0479
	Elephant 1.157	.0394

THYROID GLAND

Figure 6 shows the relationship of the thyroid gland to the body weight in this ungulate series. While the gland shows a much greater degree of variation than does the heart or brain, a certain degree of regularity is observed.

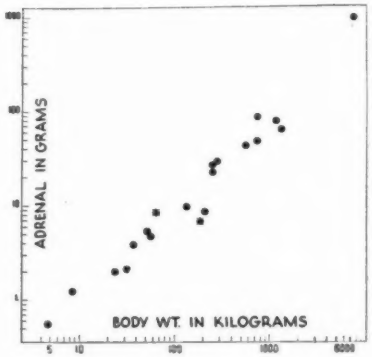


FIG. 5. Adrenal-body weight relationships showing regularity of weight increase, expressed by $y = b.x^{.666}$.

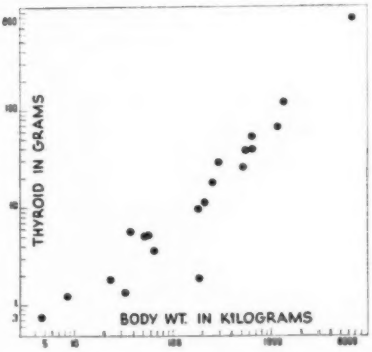


FIG. 6. Double logarithmic graph showing thyroid-body weight relationships. The values fall within a band with a slope of about .82–.89.

The ungulate thyroid gland increases at a relatively slower rate than does the body. The ratio of increase of thyroid to body is .893 according to our calculations (Fig. 6), pointing again to its relation to the body mass rather than to the body surface. Dik-dik, steinbok, impala, zebra, rhino, hippopotamus and elephant top the list so far as ratio to body weight is concerned. Reedbuck, hartebeest, waterbuck are at the bottom of the group. It may be definitely stated

TABLE IV

Organ	Mean	Standard deviation	Coefficient of C.V.
Brain	.3730± .0109	.00810± .00768	21.2% ±1.92
Heart *k* at .85	.03509± .000917	.0072± .000062	20.5% ±2.88
Adrenal	.0004453± .000003588	.000133± .0000126	29.6% ±4.76
Thyroid	.0003194± .0000243	.0001266± .0000172	39.1% ±5.30
Liver	.03964± .00121	.00970± .000876	24.4% ±2.19
Kidney	.01655± .00037	.0032± .000269	19.33% ±1.62
Lung	.01772± .00047	.00363± .00033	20.50% ±1.82

that a marked trend is discernible toward regularity, best described by the power equation or that the values fall within a well-defined band having a slope of approximately .893.

When the *b* values are calculated for this group they range from 0.0001327 to 0.0007277, the latter value found in the elephant. The mean value is 0.0003194 ±0.0000243. In view of the close relationships of many of the animals represented we assume that they stand on about the same level of a thyroidization

and that the differences simply represent individual variations. It is a striking fact, however, that the elephant stands on a considerably higher level of thyroidization than do any of the true ungulates. In view of the function of the gland its tendency to approach a direct relation with the body weight is understandable.

LIVER

The locus of points for liver plotted against body weight on double logarithmic grid (Fig. 7) shows what one might reasonably expect on the basis of functions which it performs in the animal body. It advances at almost the same rate as does the body in weight; in other words there is almost a direct correlation between liver weight and body weight, the slope of the curve being .921 for our series. In this case the liver of the elephant falls in direct line with that of lesser animals. An additional factor which makes these values plausible is the similarity of the dietary habits in this entire group, although secondary differences in the digestive tract appear which may have a specific survival value as Benedict points out in connection with ruminants and non-ruminants (P. 1 *Nutritional Physiology of the Adult Ruminant*). He states "The evolution of species suggests that the ruminant was better fitted to survive, for it exists today in a far greater number of species than non-ruminant competitors as the horse or the ass which, unlike deer, moose, caribou, and musk ox would be unable to survive a northern winter in a feral state." The warthog has relatively

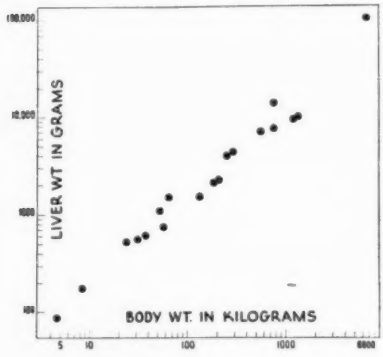


FIG. 7. Double logarithmic graph of liver plotted against body weight showing a .921 slope to the curve.

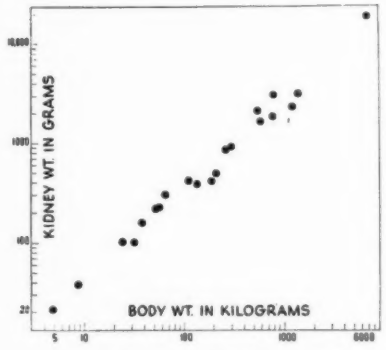


FIG. 8. Kidney-weight relationships plotted on double logarithmic grid. Slope .866.

larger liver than have the other animals. Lowest in ratio are the wildebeest, hartebeest and waterbuck.

b values range from 0.02828 to 0.06806, the latter value found in the elephant and the former in the wildebeest. The mean *b* value is 0.03964 ± 0.00121.

KIDNEY

The kidney like the liver follows the mass of the body more closely in its size

relationships than it does the surface area. When these relationships are plotted (Fig. 8) the curve has a slope of .866 with small deflections on either side of the curve. The mean b value is 0.0165 ± 0.00037. This organ shows the lowest coefficient of variation of our entire series.

LUNG

The weight of the ungulate lung stands in almost direct correlation to the body weight. A curve with a slope of approximately .976 fits the data best although a few specimens show considerable deflections from such a curve (Fig.

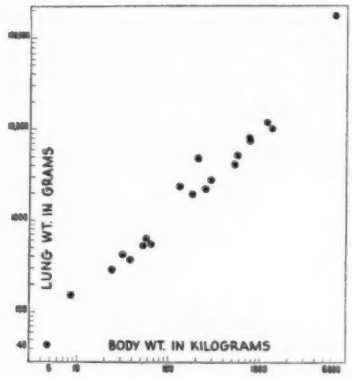


FIG. 9. Lung-body weight relationships plotted logarithmically, average slope is .967.

9). The mean b value is $0.01772 \pm .047$; the Shetland pony and the elephant show the highest values, the steinbok and the warthog the lowest.

SUMMARY

1. The body weights, brain, heart, adrenal, thyroid, liver, kidney and lung weights in twenty-eight ungulates, one hyrax and one elephant were compared by application of the power formula $Y = bX^k$.

2. When evaluated according to the power formula, the brain-body weight relationships may be expressed graphically by a line on a double logarithmic grid, having a slope of about .541. The b value of the equation, also called the cephalization coefficient, varies widely in the compared animals and this variation appears to be correlated with the degree of neural development of the animal.

3. The heart-body weight relationships when evaluated according to the power formula may be expressed graphically as a straight line on a double logarithmic grid, having a slope of about .85. The b values in this application of the equation we have termed cardiac coefficients. According to this valuation the mare, elephant, zebra, and rhino show the highest cardiac coefficients.

4. A double logarithmic graph of the adrenal-body weight relationships

causes the points to fall within a well marked band with an average slope of .866. The *b* values range from .000165 to .01157, the latter value found in the elephant.

5. The curve of thyroid-body weight value plotted logarithmically is more regular than that of the adrenal glands. A slope of .82 to .89 is observed and the thyroid coefficients (*b* values) range from .0001762 to .0007267, the latter value found in the elephant.

6. The liver and lung weights approach a linear relationship with the body weights, the *k* values being .921 for liver and .967 for lung.

7. The kidney shows a *k* value of .866 in the specimens compared and has the lowest coefficient of variation in our entire group.

8. *b* values for brain, adrenal gland, thyroid, liver, and lung are highest in the elephant. Heart *b* values are highest in the thoroughbred mare. Kidney *b* values are highest in the rhinoceros.

I wish to express my appreciation to Dr. G. W. Crile of the Cleveland Clinic Foundation for making this study possible.

REFERENCES

1. Benedict, F. G. 1932. *Yale J. Biol. & Med.* **4:** 385.
2. *Ibid.* 1936. *The Physiology of the Elephant*, Carnegie Inst. Pub.
3. Benedict, F. and R. C. Lee. 1938. Further Observations on the Physiology of the Elephant. *J. Mammal.* **19:** 175–194.
4. Benedict, F. G. and E. G. Ritzman. 1938. The Nutritional Physiology of the Adult Ruminant. Carnegie Inst. Pub.
5. Brody, S. and E. A. Trowbridge. 1937. Efficiency of Horse, Men, and Motors. *Bull. 383, Missouri Agr. Exp. Sta.*
6. DuBois, E. 1898. *Archiv. f. Anthropologie* **25:** 1–28 and 423–441.
7. *Ibid.* 1930. *Biol. Gen.* **6:** 247–291.
8. *Ibid.* 1934. *Biol. Gen.* **10:** 185–193.
9. Herrmann, G. 1929. *Proc. Soc. Exp. Biol. and Med.* **16:** 549–551.
10. *Ibid.* 1930. *Proc. Soc. Exp. Biol. and Med.* **27:** 1042–1044.
11. Hersh, H. H. 1934. *American Naturalist.* **68:** 537–561.
12. Huxley, J. S. 1932. *Problems of Relative Growth.* London, Metheun & Co.
13. Klatt, B. 1919. *Biol. Zentralbl.* **39:** 417–421.
14. Lapicque, L. 1898. *Comptes Rendus, Dix. Series, T.* **4:** 62–63.
15. Scott, W. B. 1937. *A History of the Land Mammals in the Western Hemisphere.* Macmillan, New York.
16. Snell, O. 1891. *Arch. u. Psychiatrie u. Nervenkrankheiten.* **23:** 436–446.

DEVELOPMENTAL GROWTH AND l-SERINE

By

FREDERICK S. HAMMETT AND DONALD RIVARD

The Lankenau Hospital Research Institute, Philadelphia

Aided by Grants from the Blanche and Frank Wolf Foundation, Inc., The International Cancer Research Foundation, and The Women's Auxiliary of The Lankenau Hospital Research Institute for the Promotion of Cancer Research

(*Received for publication December 14, 1938*)

Serine is of particular interest in these growth studies because it is one of the four naturally occurring amino acids in which hydroxyl replaces a hydrogen of a naturally occurring congener. As will be shown the introduction of OH here as elsewhere alters the influence of the compound in characteristic fashion.

As usual in these exploratory evaluations, the colonial-living form of the marine hydroid *Obelia geniculata* was the test animal. Details of procedure and description are referred to elsewhere (Hammett and Schlumberger '37). The l-serine was prepared, identified, and purified to the high degree requisite in these studies by Dr. Gerrit Toennies. Stock solutions containing 0.053 gram in 100 cc. fresh sea-water provided daily the aliquots from which test culture solutions were made. Two series of experiments were run with two different sets of workers. In the first or 1933 series there were 15 experiments with close to 6000 animals half of which were exposed to concentrations ranging from M/50,000 to M/5000. In the second or 1938 series there were 50 experiments with over 26,000 animals half of which were exposed to concentrations ranging from M/200,000 to M/12,500. Tests and controls were run simultaneously under like conditions of temperature, illumination and pH. Four changes into fresh sea-water were made during the 24 hour course of each experiment. The 1938 data are given in Table I. Those for 1933 are not given in order to save space. The method of derivation, construction, and analysis of the table is given in the report by Hammett and Schlumberger.

RESULTS

As in other cases so with l-serine there is a relatively ineffective concentration, a growth-affecting level, and a toxic range.

At M/200,000 test regression increase is slight. At M/100,000 and over it is greater. At M/200,000 test growth deviates from control in none of the 4 developmental brackets. At M/100,000 and over it deviates therefrom noticeably. M/200,000 concentration is therefore one of general ineffectiveness.

Apparently M/12,500 is the concentration at which the growth effect begins to be counterbalanced by toxicity. Thus, when the 4 developmental activities are combined growth is less than control in 14 and greater in but 11 of the 34 expressions at M/12,500, while of the 34 expressions at M/25,000 it is greater in 13 and less in but 11. The same mild trend is shown at the same level in the 1933 data. For whereas at the lower level test growth is better than control

in 10 and less in but 6 of the 27 expressions, at the higher level it is less in 10 and greater in but 9. The degree of harmful retardation is obviously very slight and although it does increase with increase in concentration it still remains mild as the 1933 data show. Thus even at the high M/6250–5000 concentration test growth is less than control in but 10 of the 34 expressions while it is greater in 7.

Despite the mildness and possible uncertainty of the untoward reaction at M/12,500 it seems best to use for general analysis only the data lying between

TABLE I

TREND OF TEST DEVIATION FROM CONTROL WITH RESPECT TO DEVELOPMENTAL AND METABOLIC ACTIVITY IN *l-SERINE* CULTURES. ALSO THE NUMBER OF COLONIES, HYDRANTHS AND GONOPHORES AT THE STATED CONCENTRATIONS

M/1000	200			100			50			25			12.5		
No. Expts.	10			10			10			10			10		
	Cont.	Test		Cont.	Test		Cont.	Test		Cont.	Test		Cont.	Test	
No. Colonies	150	150		149	149		150	150		150	150		150	150	
No. Hydranths	2703	2708		2721	2725		2621	2618		2595	2601		2569	2557	
No. Gonophores	414	321		288	281		334	303		461	411		337	363	
GROWTH															
	+	−	=	+	−	=	+	−	=	+	−	=	+	−	=
Initiation	2	1	4	1	5	1	1	0	6	1	3	3	4	1	2
Proliferation	3	3	2	5	1	2	5	1	2	4	3	1	4	3	1
Differentiation	4	4	2	4	2	4	4	2	3	3	4	3	1	6	3
Organization	3	3	3	3	3	3	3	1	4	5	1	3	2	4	3
METABOLISM (By Single Experiment)															
Maintenance	4	6	0	2	6	2	5	4	1	1	9	0	1	9	0
Catabolism	6	3	1	5	4	1	5	4	1	2	8	0	2	5	3
METABOLISM (By Combined Experiments)															
	Cont.	Test		Cont.	Test		Cont.	Test		Cont.	Test		Cont.	Test	
Regression	32.2	31.7		29.2	31.7		38.5	39.1		31.9	38.2		33.8	40.8	
Catabolism	85.4	85.0		79.5	79.7		84.1	85.7		82.8	80.4		84.1	81.1	
Increase in Percentage Regression															
	−1.6%			8.6%			1.6%			19.7%			20.7%		

this and the non-effective M/200,000 concentration:—those of the surely non-toxic range of M/100,000–M/50,000–M/25,000.

With the exception of regeneration, new growth initiation was held back by l-serine. The evidence is that test bud production was less than control in 8 and greater in but 3 of the 21 expressions given by the 3 sets of 1938 experiments, and that when the data are combined test is less than control in 6 and greater in but one of the 7 potentialities for initiation activity expression. Further it tended to be less in 2 and greater in none of the 3 non-toxic concentrations. The same influence was felt in the 1933 experiments.

New growth initiation by regeneration, in contrast to the group reaction as a whole, showed a trend to betterment in the presence of l-serine. Test expression of this activity was greater than control in 17 and less in 13 of the 30 experi-

ments, and it was greater in 4 and less in none of the 5 concentrations studied in 1938. In both the 1938 and the 1933 series the betterment held despite beginning toxicity.

All other developmental activities were enhanced by l-serine. Thus test proliferation was greater than control in 14 and less in but 5 of its 24 expressions, and when the data are combined it is greater in 5 and less in but one of its 8 potentialities. Further it was greater in all 3 concentrations. As for differentiation this activity was in the tests greater than control in 11 and less in but 8 of the 30 expressions, and when the data are combined it is greater in 4 and less in but 3 of the 10 potentialities. Further it was greater in 2 and less in none of the 3 concentrations. Finally organization in the tests was greater than in the controls in 11 and less in but 5 of its 27 expressions, and when the data are combined it is greater in 5 and less in but one of the 9 potentialities. Further it was greater in 2 and less in but one of the 3 concentrations. The same generalized influence was expressed in the 1933 experiments.

In non-toxic concentrations l-serine had a moderate stimulating effect on regression of the complete hydranths. Beginning toxicity had an indeterminate effect but overt toxicity gave definite acceleration. Catabolic disintegration of the senile animals was not consistently affected by the amino acid. In the 1938 series the usual retardation in toxic concentration was exhibited.

DISCUSSION

Alanine is alpha-amino propionic acid. Serine is beta-hydroxy-alpha-amino propionic acid. Replacing H by OH in the methyl group of the former produces the latter. This substitution changes the growth and metabolic influence in characteristic fashion as it did in the case of l-tyrosine and l-hydroxyproline (Hammett '36; '37).

Thus d-alanine first exhibits toxicity at M/50,000 while l-serine is not toxic until M/12,500 and then only to slight degree. And d-alanine has marked specific dynamic effect at M/200,000 while none at all is exhibited by l-serine. Indeed d-alanine enhances regression greatly at all concentrations while l-serine is but mildly active in this direction at any. And d-alanine retards initiation expression to lesser extent than l-serine, while its generalized growth enhancing influence is also less (Hammett '36).

In other words, l-serine—with its OH group—is less toxic, less active in specific dynamic factor, more inhibitive of new growth production, and a better stimulus to growth in general than is d-alanine which has no OH.

Since these same differences characterize a difference in influence of l-tyrosine from l-phenylalanine, or l-proline from l-hydroxyproline, and are always present in at least one expression when OH is potentially substituted for H in the nucleic acid derivatives (Hammett and Porter '38), the cumulative evidence would indicate that the group is of signal importance to developmental activity when it is an integral component of a naturally occurring constituent of living tissue.

Since the effect most commonly exhibited is reduction in toxicity, it may be that the other reactions stem from this rather than from participation of the

group as such therein. The cut in specific dynamic effect could be so interpreted, and the enhancement of growth influence could be taken as release from restraint. These studies can not solve this. All they can do is to establish the fact that OH in the designated combinations is important to growth and to show the products of its influence.

It is of interest to record that the low regression effect of l-serine parallels the finding of Rapport and Beard ('27) that this amino acid has no predominant specific dynamic action. When this is taken together with the fact that these workers find its congener alanine has a large specific dynamic action and I find that alanine has a large regression effect, it is evident that allocation of complete hydranth regression enhancement in non-toxic concentrations to a specific dynamic effect of the amino acid is not invalid.

In this connection it might be stated that Kisch ('32) found no consistent stimulation of respiration of rat sarcoma in Ringer's on the addition of serine thereto.

The mild but persistent enhancement of regeneration in the l-serine cultures coupled with the fact that glycine (Hammett '36a) and glycine alone shows this function to marked degree is of particular interest in the light of the following quotation from Mitchell and Hamilton ('29). "Based upon the fact established by Dakin that phenylserine gives hippuric acid in the body while phenylalanine does not, Knoop suggests that glycine originates by the oxidation of alpha-amino-beta-hydroxy acids, such as serine or an intermediary metabolite of beta-hydroxy-glutamic acid. However, the actual formation of glycine from such precursors has not been demonstrated." Although these growth studies do not show the "actual formation of glycine" from l-serine, they do show the same stimulation of regeneration as is produced by glycine, though to lesser degree of course. It is therefore not unreasonable to conclude that the regeneration effect in the l-serine experiments is due to glycine, and that Knoop's hypothesis is not an hypothesis but a statement of fact.

Sustaining evidence is that glycine direct is more effective than glycine indirect from serine. This is the expected difference since in the latter case there is loss and delay. These results are of course consistent with the finding that glycine is a specific participant in some process essential to the developmental activity of regeneration.

In a recent report of McCoy and Rose ('37) the italicized statement is made that serine is "*dispensable for growth purposes.*" This taken by itself is not a statement of fact. Serine is a participant in growth as these studies show. It is an integral component of protein. It has a function in the production of structure and material. It is not window dressing. It is essential to growth else it would not be a component of tissue. As noted long ago by Ralph Lillie ('19) "—the work of growth is derived from the energy freed in chemical reactions. Certain soluble and physically coherent products of these reactions form structure, which as soon as formed influence in a definite manner the chemical and physical processes by which further structure is formed." And serine is one of the components of this structure and of these growth processes.

Serine may not be needed in the diet of rodents. Perhaps they may be able to make this tissue constituent from other amino acids. But even here the evidence adduced by McCoy and Rose for the contention is unsure. No evidence is presented that serine may not have been formed in the gut from some of the other dietary factors. The number of animals used in the serine test was but 4. Rather a small number for such a sweeping conclusion. The period of observation was only 25 or 30 days. Rather a short time for thorough exploration. And but one concentration of the amino acid was fed with no indication that this was the optimum. My studies show beyond question that there is an optimum concentration of amino acid with which the growth influence characteristic of the compound can be elicited. And that concentrations above or below this are ineffective indices of growth influence. Finally, as I explicitly showed (Hammett '37b) and as Simmons and Todd ('38) have emphasized "Weight is not a true measure of growth—."

These results of McCoy and Rose bring no proof that serine is "dispensable for growth;" no proof that it is not essential for growth. They do show that the weight increase of 4 rats having serine in the diet is as good as if not a bit better than that of 4 rats without the added serine. It would conduce to evaluation of the amino acids in growth and nutrition if these distinctions were acknowledged.

In 1931 Jackson and Block reported that the addition of serine to the diet had but a slight effect upon growth. This record too is limited by the method of observation. The obelia data on the other hand, wherefrom several aspects of growth can be simultaneously observed, provide in this as in other instances information from which decision can be helped.

Thus in both the 1933 and the 1938 experiments the developmental activities of proliferation, differentiation, and organization were all indistinguishably forwarded by l-serine. Although each of these three major growth expressions has its own sets of reactions, participants, and determinants, all have a common denominator in those processes which leads to mass increment. "Increase in number of living units—increase in kind of living units—increase in arrangement which these living units may take—are growth expressions distinct from increase in amount of dividing differentiating and organizing substance." (Hammett '36b).

Mass increase may be either constructive or storage. From constructive increase derives expansion of functional and or structural capacity. This is obviously what happens in the presence of l-serine. The sum total of the processes productive of this increase is called anabolism. The conclusion is inescapable that l-serine is a specific determinant of some process essential to constructive mass increment or anabolism.

Consistent is the absence of forwarding action on the developmental growth activity of initiation. For here the processes are more preparatory than accretive; less concerned with mass increment than with laying a foundation for developmental change in substance already present: Not so much concerned in developing towards functionableness as developing towards development.

The forwarding of regeneration is no great obstacle to this interpretation for this growth expression depends upon renewal of substance lost through injury as well as on reconstitution of the material injured. And anabolism is essential for renewal.

The foregoing analysis indicates that the obelia reaction to l-serine is peculiar to this amino acid. This individuality of influence is subtantiation of the premise that each of the naturally occurring tissue components of general distribution has its own part to play in developmental growth.

SUMMARY AND CONCLUSION

From 65 experiments with close to 32,000 animals half of which were exposed to l-serine in concentrations ranging from M/200,000 to M/5000 in two different years with two different sets of workers, it was found that:—

1. Initiation activity was retarded.
2. Regeneration was favored.
3. Proliferation, differentiation, and organization expression were forwarded.
4. Catabolism was not specifically affected.
5. Maintenance was slightly reduced and regression increased in the higher concentrations.
6. Toxic action on growth was very mild and began at M/12,500.
7. l-Serine, like all the other compounds so far studied, produces an effect which is individual and characterizing.

Comparing the data with those for the non-hydroxyl carrying alanine it is seen that here as in the other cases where methyl H has been replaced by OH there is reduction in toxicity; reduction in specific dynamic effect; increased retardation of new growth initiation; and increase in expression of growth influence. Thus the evidence that OH as found in these naturally occurring tissue components of general distribution is a definitive factor in growth and metabolism is becoming more and more evident.

The fact that regeneration is favored by l-serine supports the concept of Knoop that this amino acid is broken down by beta-oxidation to glycine, since previous work shows indubitably that glycine acts specifically to forward this particular growth activity.

Since constructive mass increment is the basis for expansion of functional and structural capacity and since l-serine acts to forward all phases of developmental growth save that which pertains to preparatory processes, the conclusion is drawn that this amino acid is a specific contributor to some process essential to anabolism.

REFERENCES

HAMMETT, F. S. 1936. Comparison of d-alanine, l-phenylalanine, and l-tyrosine with respect to their participation in developmental growth. *Protoplasma*, **27**: 52–60.

HAMMETT, F. S. 1936a. The regeneration favoring activity of glycine. *Protoplasma*, **27**: 65–68.

HAMMETT, F. S. 1936b. *The Nature of Growth*. Science Press and Printing Co., Lancaster, Pa. 66 pp.

HAMMETT, F. S. 1937. The role of l-hydroxyproline in developmental growth. This Journal, **1**: 291–298.

HAMMETT, F. S. and H. SCHLUMBERGER. 1937. The role of l-aspartic acid in developmental growth. This Journal, **1**: 68–77.

HAMMETT, F. S. 1937a. Nutrition vs. Growth. *Science*, **86**: 560–561.
HAMMETT, F. S. and M. R. PORTER. Developmental growth and nucleic acid components VIII. Xanthine. This Journal, **2**: 71–80.
JACKSON, R. W. and R. J. BLOCK. 1931. Metabolism of cystine and methionine. *Science*, **74**: 414.
KISCH, B. 1932. The influence of amino acids on respiration. IV. Experiments with serine, valine, carnosine, isoleucine, and phenylamino acetic acid. *Biochem. Z.* **244**: 451–458.
LILLIE, R. S. and E. N. JOHNSTON, 1919. Precipitation structures simulating organic growth. II. A contribution to the physio-chemical basis of growth and heredity. *Biol. Bull.* **36**: 225–272.
MCCOY, R. H. and WM. C. ROSE. 1937. The relation of glycine and senile growth. *Jour. Biol. Chem.* **117**: 588–591.
MITCHELL, H. H. and T. S. HAMILTON. 1929. *Biochemistry of the Amino Acids*. Chem. Catalog Co., N. Y.
RAPPORT, D. and H. H. BEARD. 1927. The effects of protein split products upon metabolism. II. The individual amino acids of fraction 1 of the butyl alcohol extraction, and their relation to the specific dynamic action of protein. *Jour. Biol. Chem.* **73**: 299–319.
SIMMONS, K. and T. W. TODD. 1938. Growth of well children: Analysis of stature and weight, 3 months to 13 years. This Journal, **2**: 93–134.

SULPHUR AND GROWTH STIMULATION*

(A consideration of larval preparations)

By
SEWARD E. OWEN

From the Cancer Research Unit, Veterans' Administration, Hines, Illinois

This report deals with a chemical study of the natural sulphur complexes known to stimulate growth. Special attention is given the larval derivatives.

The sulphur containing amino acid cystine is necessary for growth. Animals including man, reputedly cannot synthesize this amino acid. While cystine and its derivatives and complexes are essential for the biological activity of growth, there are other compounds containing sulphur which are endowed with direct or indirect growth stimulating properties. The problem is of interest from the viewpoint of malignancies as it may be accepted that growth processes are stimulated in the development of cancer, although not all stimulations to growth result in cancer, Loeb (20), Owen (29) and others.

The importance of sulphydryl in cell proliferation was first shown by Hammett (9). He (10) has reported cysteine (CSH), cystine (CSSC) and glutathione (GSH) as accelerating cell multiplication. Additional studies on the sulphydryl (SH) compounds as stimulating growth has been done by Reimann (34), Hammett and Reimann (11), Brunstig and Simonsen (4), Livingston (19) and Owen (29).

The early foundations of similar studies exist in the work of Hopkins (14) who accepting the findings of de Rey-Pailhade (36), Morner (27), Heffter (13) and Arnold (1), isolated and studied the properties of an auto-oxidizable constituent of the cell. This constituent was later synthesized by Quastel et al. (33) and by Stewart and Tunnicliffe (39). The substance was glutathione and proved identical with the natural product. Applied studies regarding the healing properties of maggot larva were reported by Baer (2). Livingston and Prince (18) first used larval derivatives in the treatment of chronic wounds.

The reversible oxidation-reduction systems of cysteine-cystine and of reduced and oxidized glutathione have been extensively studied by Kendall and Nord (16). They give evidence that cysteine and cystine alone cannot form a reversible oxidation-reduction system, but that an intermediate oxygen addition product in its oxidized and reduced form does constitute such a system. The ratio of cysteine to cystine determines the absolute value of the oxidiation-reduction potential at the equilibrium point. Hopkins (14) has shown that fresh tissues are capable of reducing the oxidized form of glutathione. Kendall and Holst (15) and Shinohara and Kilpatrick (37) state that when any thiol compound is mixed with cystine, the characteristic color of the cysteine complex is produced by the nitro-prusside reaction. Mirsky and Anson (26) have shown that cystine (CSSC) oxidizes all the SH groups and no other groups of

* Published with the permission of the Medical Director of the Veterans' Administration, who assumes no responsibility for the opinions expressed or the conclusions drawn by the author.

the proteins. They utilize this in a test for the presence of SH groups in proteins. Shinohara and Kilpatrick (loc. cit) found that cystine undergoes hydrolysis on standing to produce cysteine. An alkaline solution of cystine undergoes similar changes at a much faster rate. Cysteine solutions change gradually on standing, mainly owing to oxidation. Shinohara (38) using a solution of .01 M cysteine in .2 M HCl has given the following formula for the rate of oxidation: $C_0(1-.0023\,N)=C$ where C_0 is the initial concentration of cysteine (.01 M) and C is the cysteine concentration after N days preservation. The rate of cystine (CSSC) to cysteine (CSH) is quite slow as we have tested solutions at least two years old and still obtained positive evidence of cystine.

There are many methods for the determination of thiol and disulphide compounds, those generally utilized are listed: (a) the nitro-prusside reaction of Arnold (1), (b) the iodine or iodate procedure, Okuda (28), Baernstein (3), Lucas (21), and Virtue and Lewis (44), (c) the phosphotungstate acid method of Folin and Looney (6), Folin and Marenzi (7) and Lugg (22), (d) the napthoquinone sulphonate test, Sullivan (40), Prunty (32), Lugg (23) and Bushill et al. (5), (e) cuprous oxide precipitation technique of Vickery and White (42) and Pollard and Chibnall (31), (f) the cobalt complex reaction, Michaelis and Barron (24) and Michaelis and Yamaguchi (25) and Kendall and Holst (loc cit.), (g) the cystine reduction procedure Mirsky and Anson (loc. cit.).

For our use we have selected the following procedures as the most promising, a modification of the nitro-prusside reaction, the phospho-tungstate procedure and the cobalt complex test method. The remaining tests according to reports lack specificity, are inaccurate or exceedingly tedious of performance. A good limit of accuracy seems to be about .482 milligram per liter for cysteine. This is considerably above the minimum effective concentration of SH necessary for growth stimulation which according to Hammet (12) is 2×10^{-7}.

METHODS

The nitro-prusside test used was that of Shinohara and Kilpatrick (loc. cit.) with a slight alteration in manipulation. The minimum concentration of cysteine which can be detected by this test is approximately 4×10^{-5} M. Procedure is as follows: Two cc. of a .2 M zinc acetate solution is added to 5 cc. of the specimen. The mixture is shaken lightly and two cc. of 1 M ammonium hydroxide are added. The mixture is shaken or mixed well and .5 cc. of a 5% sodium nitroprusside solution is added. A pink coloration to the precipitate denotes the presence of the SH group characteristic of cysteine. If following the addition of the zinc acetate and the ammonia, the mixture be centrifuged before the addition of the nitroprusside so as to overlay the precipitate and underlay the supernatant liquid, the gradual development of a pink ring within the precipitate will be noted. This is in contrast to the lower precipitate and is easily recognized. Very little pink color is imparted to the supernatant solution.

The cobalt complex test was done as follows: two cc. of a. 2 M cobaltous chloride ($CoCl_2\cdot6H_2O$) solution were added to five cc. of the specimen. The tube is rotated to mix the contents and 4 cc. of .5 N sodium hydroxide solution

is added. The tube is now agitated to mix and 1 cc. of hydrogen peroxide (3%) is added. Rotate the tube to mix the contents. When the major part of the activity has ceased centrifuge or filter to remove the cobaltic hydroxide. The presence of cysteine is indicated by a brownish yellow color of the supernatant solution. The color is stable for long periods. Pyruvic acid, thiourea, thioacetic acid, the thio alcohols and the thiophenols are negative. The brown color given by the thioacids fades on standing overnight. Glutathione produces a brownish pink color and practically no color before the peroxide is added. Histidine gives a brownish color but on oxidation by air or peroxide a pink results. Hydrogen sulphide leaves the solution colorless. If cystine be added to a solution containing a thiol compound and the test then made the characteristic color for cysteine will appear. The atypical color produced by cystine and hydrogen sulphide is said to be easily recognized. As is shown later the alkalinity of the specimen solution must be considered.

The phospho-tungstate procedure was carried out as follows: Five cc. of the specimen solution are added to a mixture containing 10 cc. of 2 M sodium acetate and 3 cc. of 2 M acetic acid. Four cc. of the phospho-18-tungstic acid reagent (Folin and Marenzi loc. cit.) are next added. The presence of cysteine is indicated by a blue coloration of the liquid. If quantitative determinations are made each test mixture may be made to fifty cc. and compared to a cysteine standard. The standard solution is quite unstable. If the specimen solution be reduced by the addition of one cc. of saturated sodium sulphite solution before testing the cystine present is changed to cysteine which may subsequently be tested for, or after obtaining the test for actual cysteine the sulphite be added to the mixture the color is deepened if cystine is also present.

RESULTS

The findings, obtained by employing these tests on various chemicals, larval preparations and growth stimulating agents, are given in Table A.

DISCUSSION

The findings of the tests indicate that in general the results obtained with the modified nitro-prusside reaction and the phospho-tungstate procedure are parallel. This is especially true as regards cysteine. The nitro-prusside test used, however, did give positive results with neutral or with acid solutions of oxidized glutathione while the phospho-tungstate test was negative on these. The nitro-prusside test for the SH groups gives false positives with the resorcinols. The phospho-tungstate test was negative with these chemicals unless the test was modified to serve for the SS groups when the latter test also gave false positives. The cobalt-complex test procedure as used seems not to be specific for the SH groups as the alkalinization employed in the procedure apparently changes the SS groups to SH groups thus to give false positives. It appears that the cobalt-complex test is a better one for the SS groupings then for the SH groups. The phospho-tungstate procedure appears to be the most reliable one for sulphydryl groups.

The indications as to cysteine content of larval extracts are that this substance doesn't exist in amounts (greater than 4×10^{-5} M) sufficient for the chemical tests to indicate its presence. These same extracts, however, cause wound healing, Livingston loc. cit. Cysteine is testable in fresh larval extracts and for some time in stabilized preparations. Cystine does exist in older larval preparations adjusted so as to be on the acid side.

TABLE A

SHOWING THE RESULTS OBTAINED BY THE THREE CHEMICAL TESTS FOR SH AND SS GROUPINGS, ON VARIOUS MATERIALS AND ON GROWTH STIMULATING PREPARATIONS

# Type substance used	Nitroprusside	Cobalt-complex	Phosphotung (SH)	Phosphotung. Sulphite (SS)
1. Water	—	—	—	—
2. Cystine, neutral soln.*	—	+	—	+
3. Cystine, acid soln.	—	+?	—	+
4. Cystine, alkaline	—	+	+	+
5. Cysteine HCl, soln.	+	+	+	0
6. Cysteine, Carbon treat.	+	+	+	0
7. Carbon water filt.	—	—	—	—
8. Cystine soln. acid boiled	+	+	+	+
9. Cystine soln, alk. boiled	+	+	+	+
10. Glutathione soln, (GSSG)	+	+	—	+
11. Crushed larva fresh.	+	+	+	+
12. Dessicated larva	+	+	+	+
13. Aqueous larval ext.	—	+	—	+
14. Aq. larval ext. Zn-acid treat.	+	+	+	+
15. Aq. larval ext., SO_3 reduced	+	+	+	0
16. Liquid larval ext.	—	+	—	+
17. Larva-agar gel	+	+	?	+
18. Liquid larval ext., old	—	+	—	+
19. Larva-gel complex	+	+	+?	+
20. Agar gel only	—	—	—	—
21. Complex gel only	—	—	—	—
22. Larva ext.-serum	?	+	—	+
23. Serum only	?	+	—	+
24. Larva ext -serum incubate	?	+	—	+
25. Autoclaved larva extract	—	+	—	+
26. Dessicated liver	+	+	+	+
27. Fresh yeast ext.	+	+	+	+
28. Yeast ext. aged	—	+	—	+
29. Resorcinol 1:1000 soln.	+	+	—	+
30. Hexyl resorcinol 1:1000 soln.	+	+	—	+
31. Glutathione soln. (GSSG) acid	+	+	—	+
32. Glutathione soln. (GSSG) alk.	+	+	+	+
33. Glutathione (GSSG) SO_3 treat.	+	+	+	+

Liquid and gel larval extracts are 5% fresh larva. The chemicals were used in approximately 10 mgms. per 100 ccs. solution. Carbon treat. is carbon clarification by .5 gm. carbon per 100 cc. soln.

Since the sulphydryl group has been reported as essential to growth stimulation it is of interest to know that larval preparations containing insufficient SH to give chemical tests, do stimulate growth of tissues. Since larval preparations in contact with serum at body temperature does not release testable SH effects the probable mechanism of growth stimulation by these preparations must be through other channels. The possibilities are (a) the larval preparations do contain SH groups but these are in concentrations too dilute to pick up by chemical tests. This agrees with the biological findings of Hammett (loc. cit.) or

(b) the appropriate oxidized form namely SS is changed to the effective SH form on contact with the tissues, this agrees with the findings of Hopkins loc. cit., with the studies of Anson and Mirsky loc. cit. and with the report of Kendall and Holst loc. cit. For cystine to act stimulatingly in healing processes it would seem that the conditions in the wounds should be reducing in nature. Similar effects might be accomplished by the alkalinity of the tissues changing the acid solutions of cystine so as to promote the formation of cysteine. It would appear that cysteine need not be in detectable concentrations in growth stimulating preparations. It should, however, be available in the wound from precursors existing in the applied extracts. (c) Since cystine and cysteine or reduced and oxidized glutathione do set up reversible oxidation-reduction systems and since the rate of oxidation of cysteine to inactive agents seems faster than the rate of cysteine formation from cystine, it would appear that this system would be in consonance with the biological facts, viz., the findings of Hammett (10) that cystine does stimulate growth, with the writings of Reimann (35) who assigns biological activity to the levo form of cystine and with the clinical results of Livingston (19) on over a thousand clinical cases where materials containing considerable SS groupings but no testable SH groups, were effective.

Thompson and Voegtlin (41) have shown that the glutathione content of normal animal decreases with age. This seems to parallel the animals' ability for tissue repair or growth of injured tissues. Voegtlin and Thompson (43) also mention that actively growing tissues may give a negative Sullivan test for cysteine or cystine. They believe this indicates a fixed SH group in the tissues similar to the findings of Hopkins loc. cit. The minimal effective concentrations of sulphydryl, however, for healing or growth stimulation, are beyond the reach of the available chemical tests, Hammett loc. cit. A minimal positive chemical test for SH may indicate about one hundred times the biological effective concentration. In the healing of wounds unwanted overgrowth has been noted by larval preparations so concentrated as to give consistent positive chemical tests for SH Livingston (17).

Many problems immediately arise for consideration in the study of chemical changes of biological growth processes. In chronic wounds is the tissue sulphydryl more fixed or less inactive than in healing wounds? Is the supply of the levo form of the sulphydryl chemicals low in chronic wounds? Is the lack of healing associated with a change in the oxidation-reduction potentials? Might the tissue enzymes be in imbalance in non-healing wounds? There are countless other possibilities. A preliminary study of the oxidation-reduction potentials of wounds is under investigation. Correlation of our findings with the healing rates will be offered in a later paper. A further consideration of the oxidation-reduction situation in malignancies will be made.

SUMMARY

1. A series of chemical tests for the SH group on growth stimulating preparations have been made.

2. Active growth stimulating preparations may not give positive chemical

evidence of the sulphydryl group by any of the three methods employed.

3. Probable explanations are offered why materials so low in sulphydryl content, as to give negative chemical tests, do stimulate the healing rate of wounds.

REFERENCES

1. ARNOLD, V. 1911. Eine farbenreaktion von eiweisskorpern mit nitroprussidnatrium. *Zeitschr. physicl. Chem.* **70**: 300–301.
2. BAER, W. S. 1931. The treatment of chronic osteomyelitis with the maggot (larva of the blow fly). *Jour. Bone and Joint Surg.* **13**: 438.
3. BAERNSTEIN, H. D. 1930. Gasometric determination of cysteine and cystine, *Jour. Biol. Chem.* **89**: 125–131.
4. BRUNSTIG, L. S. and SIMONSEN, D. C. 1933. Cutaneous ulcers treated by the sulphydryl containing amino acid cysteine. *Jour. Am. Med. Assn.* **101**: 1937–1940.
5. BUSHILL, J. H., LAMPITT, L. H. and BAKER, L. C. 1934. Determination of cystine. Use of Zeiss photometer. *Biochem. Jour.* **28**: 1293–1304.
6. FOLIN, O. and LOONEY, J. M. 1922. Colorimetric methods for separate determination of tyrosine, tryptophane and cystine in proteins. *Jour. Biol. Chem.* **51**: 421–434.
7. FOLIN, O. and MARENZI, A. D. 1929. The preparation of uric acid reagent completely free from phenol reagent. *Jour. Biol. Chem.* **83**: 109–114.
8. GIROUD, A. and H. BULLIARD. 1934. Mise en evidence des substances a fonction sulphydryl. *Bull. Histol. Appl.* **11(4)**: 169–172.
   See also Arnold for reference to this paper.
9. HAMMETT, F. S. 1929. The natural chemical stimulus essential for growth by increase in cell number. *Protoplasma* **7**: 297–372.
10. HAMMETT, F. S. 1933. Growth retardation by the partially oxidized sulphydryl of cysteine. *Science* **77**: 190–191.
11. HAMMETT, F. S. and REIMANN, S. P. 1929. The proliferative response to sulphydryl in mammals. *Jour. Exp. Med.* **50**: 445–448.
12. HAMMETT, F. S. Personal communication.
13. HEFFTER, A. 1908. Die reduzierenden bestandeile der zellen. *Med. Naturwiss. Arch.* I.81 (Maly's Jahresb. 1908).
14. HOPKINS, F. G. 1921. On an autooxidizable cell constituent. *Biochem. Jour.* **15**: 286–305.
15. KENDALL, E. C. and HOLST, J. E. 1931. The oxidation of cobaltous cysteine. *Jour. Biol. Chem.* **91**: 435–474.
16. KENDALL, E. C. and NORD, F. F. 1926. Reversible oxidation-reduction systems of cysteine-cystine and reduced and oxidized glutathione. *Jour. Biol. Chem.* **69**: 295–537.
17. LIVINGSTON, S. K. Personal communication.
18. LIVINGSTON, S. K. and PRINCE, L. H. 1932. The treatment of chronic osteomyelitis with special reference to the use of maggot active principle. *Jour. Am. Med. Assn.* **98**: 1143.
19. LIVINGSTON, S. K. 1937. Therapeutics of maggot active principle. *Am. Jour. Surgery.* **35**: 554–556.
20. LOEB, L., BURNS, E. L., SUNTZEFF, V. and MOSKOP, M. 1937. Sex hormones and their relation to tumors. *Am. Jour. Cancer.* **30**: 47–53.
21. LUCAS, C. C. and KING, E. J. 1931. Eine bemerkung zur iodometrischen titration des glutathions. *Biochem. Ztschr.* **235**: 66–69.
22. LUGG, J. W. H. 1932. The application of phospho-18-tungstic acid (Folins reagent) to the colorimetric determination of cysteine, cystine and related substances. *Biochem. Jour.* **26**: 2144–2150.
23. LUGG, J. W. H. 1933. Sullivan's reaction for the quantitative determination of cysteine and cystine. *Biochem. Jour.* **27**: 668–673.
24. MICHAELIS, L. and BARRON, E. S. G. 1929. Oxidation-reduction systems of biological significance. Comparative study of complexes of cysteine with metals of the iron group. *Jour. Biol. Chem.* **83**: 191–210.
25. MICHAELIS, L. and S. YAMAGUCHI. 1929. Composition of oxidized cobalt complex of cysteine. Colorimetric method for micro-analysis of cobalt. *Jour. Biol. Chem.* **83**: 367–373.
26. MIRSKY, A. E. and ANSON, M. L. 1935. Sulphydryl and disulphide groups of proteins, methods of estimation. *Jour. Gen. Physiol.* **18**: 307–323.
27. MORNER, K. A. H. 1904. Zur kenntnis der spallungsprodukte des cystins. *Ztschr. Physiol. Chem.* **42**: 349–364.

28. Okuda, Y. 1925. New method for the determination of cystine in proteins. *Jour. Biochem. Japan.* **5**: 217–227.
29. Owen, S. E. 1937. Sulphydryl and radon induced necrosis. *Growth* **1**: 130–134.
30. Owen, S. 1938. The genesis of cancer. *The Military Surgeon* **82**: 218–225.
31. Pollard, A. and A. C. Chibnall. 1934. The proteins of grasses III. The cystine content of certain grasses and other pasture plant proteins. *Biochem. Jour.* **28**: 326–331.
32. Prunty, F. T. G. 1933. The determination of cystine in biological material. *Biochem. Jour.* **27**: 387–389.
33. Quastel, J. H., Stewart, C. P. and Tunnicliffe, H. E. 1923. On glutathione constitution. *Biochem. Jour.* **17**: 587–592.
34. Reimann, S. P. 1931. The hyperplastic reaction of the skin to sulphydryl and its significance in neoplasia. *Amer. Jour. Cancer* **15**: 2149–2168.
35. Reimann, S. P. 1934. Practical aspects of sulphydryl. *Cyclopedia of Medicine* (Piersol) E. A. Davis Co. Phila.
36. De Rey Pailhade, J. 1888. Sur un corps d'origine organique hydrogenant le soufre a froid. *Compt. Rend. Acad. Sci.* **106**: 1683–1684.
37. Shinohara, K. and Kilpatrick, M. 1934. The stability of cystine in acid solution. *Jour. Biol. Chem.* **105**: 241–251.
38. Shinohara, K. 1935. The determination of thiol and disulfide compounds with special reference to cysteine and cystine. *Jour. Biol. Chem.* **109**: 665–679.
39. Stewart, C. P. and Tunnicliffe. H. E. 1925. Glutathione synthesis. *Biochem. Jour.* **19**: 207–217.
40. Sullivan, M. X. 1926. Suppl. 1929. A distinctive test for cysteine. *U. S. Pub. Health Reports. U. S. P.H.S.* **41**: 1030, also supplement 78. 1929.
41. Thompson, J. W. and Voegtlin, C. 1926. Glutathione content of normal animals. *Jour. Biol. Chem.* **70**: 793–800.
42. Vickery, H. B. and White, A. 1933. The use of cysteine cuprous mercaptide in the determination of cystine. *Jour. Biol. Chem.* **99**: 701–715.
43. Voegtlin, C. and Thompson, J. W. 1926. Glutathione content of tumor animals. *Jour. Biol. Chem.* **70**: 801–806.
44. Virtue, R. W. and Lewis, H. B. 1934. Iodometric determination of cystine in urine. *Jour. Biol. Chem.* **104**: 415–421.

ON THE NATURE OF ADAPTIVE ENZYMES

By
OTTO RAHN
From the Laboratory of Bacteriology, Cornell University

(*Received December 29, 1938*)

The purpose of this paper is to show that adaptive enzymes are not an exceptional biological phenomenon, but that they belong to a set or class of quite remarkable chemical reactions displayed by most cells in a changing environment. Their peculiarity consists in four points: (1) the reaction does not take place unless it is needed; (2) the mechanism necessary to produce this reaction is not preformed in the cell, but is formed only when needed, (3) the mechanism is not inherited, but the ability to set up this mechanism in case of need is inherited, (4) all reactions involved are highly specific, chemically speaking.

The credit of having established the most important facts about adaptive enzymes belongs to Karström (1930, 1938). If the examples here are taken from the recent publications of Hegarty (1938, 1939), it is because the experiments described later have been made by exactly the same technique. Hegarty grew *Streptococcus lactis* in glucose broth, separated the cells from the medium by centrifugation, and re-suspended them in a phosphate buffer (pH 7.0) plus glucose or plus galactose, in such concentrated suspensions that growth was excluded. A parallel set of suspensions was made with cells grown in galactose broth. The lactic acid which was formed very quickly in these concentrated suspensions was measured by titration, and the result, expressed in mg. lactic acid per 100 cc., of suspension, was as follows:

		1 hr.	2 hrs.	3 hrs.	4 hrs.
Cells grown with glucose, suspended in	glucose	240	360	420	470
	galactose	0	0	0	0
Cells grown in galactose, suspended in	glucose	160	300	370	420
	galactose	180	270	400	410

The cells grown with glucose for 12 hours cannot ferment galactose while those grown with galactose ferment both sugars. Glucose is always fermented, even when the bacteria had been grown without any sugar; the enzyme is "constitutive." But galactozymase (the enzyme forming lactic acid from galactose) is a typical adaptive enzyme, being formed only in cells grown in the presence of galactose.

Hegarty showed later (1939) that very young cells may adapt themselves without growth, but that some time is required for this adaptation. A culture 12 hours old, such as was used in the experiment above, must be considered old, or at least "adult." When cells are taken from a glucose broth culture 1 hour after being inoculated, and are then placed in galactose, they will begin to form lactic acid after 1.5 hours. Cells from a 2 hour culture require 3 hours for

adaptation, and this time increases as the culture becomes older. It also varies with the kind of sugar as may be seen from the following data where the formation of lactase seems much more difficult than that of galactozymase:

Cells grown in glucose

for	1	hour,	ferment	galactose	after	1.5	hrs.,	lactose	after	8 hrs.
"	2	"	"	"	"	3	"	"	"	9 "
"	3	"	"	"	"	7	"	"	"	15 "
"	4	"	"	"	"	9	"	"	"	16 "
"	6	"	"	"	"	13	"	"	"	20 "
"	8	"	"	"	"	13	"	"	"	20 "
"	12	"	"	"	"	13	"	"	"	20 "

The most important factor in adaptation seems to be the physiological age of the cells when they come in contact with the new substrate. The older they are, the slower the adaptation. The delay is needed to construct the mechanism which produces the galactozymase or lactase. The establishment of this mechanism is possible only in the presence of galactose, or lactose.

The next step was to ascertain experimentally what becomes of this mechanism, and of the enzyme as such, in the absence of galactose. Cells of the same strain as above were grown in lactose broth, separated from the medium by centrifugation, and suspended in 10 times as large a volume of glucose broth in which the cells multiplied 20-fold in 8 hours. Every two hours, a sample of this culture was centrifuged, and the cells were placed in phosphate buffer plus glucose, or plus galactose, or plus lactose to ascertain the intensity of fermentation. The result was an increasingly rapid fermentation of glucose, but a decreasing rate for lactose and galactose if computed per cell per hour (table I). As the plate counts show, experiment I was disturbed during the first hours by a bacteriophage which destroyed the vitality of the cells earlier than their enzymes, and thus caused an impossibly high glucose-fermenting capacity to appear after 4 hours.

TABLE I

GALACTOSE-GROWN CELLS AFTER TRANSFER TO GLUCOSE BROTH

		Experiment I					Experiment II				
		Age of glucose broth culture					Age of glucose broth culture				
		0 hrs.	2 hrs.	4 hrs.	6 hrs.	8 hrs.	0 hrs.	2 hrs.	4 hrs.	6 hrs.	8 hrs.
Plate count, in million cells per cc.		52.5	111	86	586	1320	54	149	456	482	1360
Fermenting Capacity (10^{-10} mg. lactic acid per cell per hour)											
cells suspended in	glucose	4.8	23.8	153.0	19.1	6.6	8.1	11.5	18.3	22.6	25.5
	galactose	3.0	2.2	2.6	0.3	0.11	5.4	1.8	0.14	0.11	0.22
	lactose	2.0	1.1	1.2	0.13	0.08	7.1	?	0.37	0.21	0.14
Potential acid formation of all cells per liter of culture, mg. per hour,											
if suspended in	glucose	25	264	1330	1120	875	23.4	187	973	894	728
	galactose	15.9	24.5	22.0	16.0	15.0	15.6	28.6	7.7	4.2	6 to 9
	lactose	10.3	12.5	10.0	7.5	10.0	20.7	?	19.6	8.2	3 to 6

The increase in glucose was to be expected. But the cells had possessed some adaptive enzymes at the moment of separation from the lactose broth, and also the mechanism to form lactase and galactozymase. If the enzymes remained unchanged, the acid formed per unit volume of the culture (regardless of the number of cells) should remain constant. If the enzyme-producing mechanism continued to function, the acid per unit volume should increase. Table I shows that the potential acid from lactose and galactose per liter of culture remained essentially constant in the first experiment, and slowly declined in the second. An exception is the increase of galactozymase in the 2 hour culture. This was due to renewed adaptation of the very young cells which was evident by the fact that the rate of galactose fermentation by these cells increased from hour to hour, while they retained a constant rate with glucose fermentation. This indicates that the enzymes as such deteriorate but slowly, and are probably distributed among the offspring of the dividing cells so that ultimately the amount of enzyme per cell is negligible.

The mechanism to produce the enzyme apparently does not function in the absence of the specific substrate. It may become distributed in cell division like the enzymes, but it may perhaps consist of only one indivisible unit which can not be distributed by cell division, and would remain as an entity in one cell while the twin cell would be altogether without it. This latter possibility is in good agreement with the fact that no increase in adaptive enzymes is noticeable when the cells are re-suspended in galactose or lactose. The enzyme-producing mechanism has not multiplied.

While thus the loss of adaptive enzymes is understandable to a certain degree, we are still entirely unable to explain how an enzyme can be formed only in the presence of a substrate and not in its absence.

If we look around for analogies for this unusual type of chemical reaction, we may be surprised to find that a very similar type of reactions has been studied very extensively for more than 50 years, namely the formation of antibodies, such as antitoxins, precipitins, agglutinins, and the compounds resulting in allergies and in anaphylaxis. About the entire group of reactions, Gay (1935, p. 333) made the following statement:

"Whatever the mechanism of antibody production may be, it is clear that the appearance of freely circulating antibodies as well as the divers manifestations of altered cellular function represent the sum total of an effort on the part of the body to adapt itself in a specialized manner to a given injurious stimulus. This positive response may be regarded as one of the most primitive functions of the living cells of all biological forms, phenomena of increased resistance having been successfully demonstrated with the lowest animal forms, possibly even in plants and unicellular organisms."

This statement may now be extended to include adaptation to certain beneficial stimuli, namely food compounds, by formation of adaptive enzymes.

Let us see how far this analogy goes:

In a normal animal, the body cells do not produce antibodies. Some less specific bactericidal or agglutinating compounds of blood or tissues may be

compared with constitutive enzymes. Only after the antigen has been injected, does the production of antibodies begin. In the same way, the normal, untrained bacteria do not produce the special enzyme. Only after the cells have come in contact with the specific substrate, do they begin to produce the adaptive enzyme. In both cases, we have the potential ability of the cells to produce a certain compound, but the special stimulus is necessary to start production.

The delay of several hours required by bacteria to adapt themselves to a new substrate corresponds to the "incubation period" in animals. Usually 2–3 days are required after injection before the antibodies can be detected. This seems long compared with the few hours observed in bacteria, but the rate of metabolism and growth in bacteria is very much greater, and we use the hour as time unit while in the feeding and growth of animals, the day is the unit.

Formation of antibodies ceases after a while if only one injection is given. This is because all antigen is eliminated from the body. But the cells have not "forgotten." We must not compare these body cells with bacteria rapidly multiplying in a medium without the specific substrate, but rather with resting bacteria. Even a year after the injection, many of the cells which had learned to produce the antibody are still present in the animal, and they still possess the mechanism to do it. A second injection results therefore in a very rapid reaction, sometimes so rapid as to produce anaphylaxis.

One more important agreement is the great specificity in both mechanisms. The enzyme fits exclusively the one substrate which stimulated its production, and the specificity of antibodies is almost proverbial.

There is one striking difference. In bacteria, the adaptation is most rapid and complete in the youngest cells, but the very young animals produce decidedly less antibodies than adults. This may be explainable by differences in the physiological condition of the macrophages (histiocytes), in their rate of elimination or reproduction, but so little is known about these cells that no statement can be made.

Analogies in Differentiation: The undifferentiated cells of the earliest embryonic stages become differentiated through the action of certain compounds, the organizers. Thus, prospective nerve tissue, skin tissue etc. become established. At the very earliest stage, prospective nerve tissue transplanted to belly ectoderm becomes ectoderm. Only a short time later in development, however, an eye-Anlage transplanted to the tail of a tadpole results in the formation of a rudimentary eye. These older cells seem comparable to a bacteria culture 12 hours old, both of which are still multiplying, but already set in their way and no more capable of adaptation.

So far, it may well be that the analogy holds, and that the chemical mechanism of earliest differentiation is similar to the formation of antitoxins or adaptive enzymes. We have, at the start, cells potentially capable of a certain reaction, but not performing it without a specific stimulus. When the stimulus comes, a certain time is required for the adaptation to it. When the reaction manifests itself, it is absolutely specific. But it seems as if the analogy ends here.

At this stage, other differentiation factors produced by the cells themselves

or by environmental tissues affect the cells further, and the result is the final establishment of true tissues with "irreversibly determined" cells. It is this irreversibility which does not fit in the analogy, for the formation of adaptive enzymes is reversible, and so is the antibody formation, although more slowly. It may be, of course, that the further specialization of the cells which takes place after the task of the original organizer is ended, blocks the way for reversal.

Certain adult tissues require hormones for the normal performance of their functions. While we know the normal and the abnormal functions, we do not understand how the hormone exerts its control over the function, and hence, it is useless to speculate on the mechanism involved.

Analogy with Learning. If we define learning as formation of associations, it shows a close parallelism to the phenomena just mentioned. A dog meeting a certain man for the first time forms an association between the man's scent and his personality. How such association is formed, is not known, but to the biochemist, it does not sound so impossible to assume it to be some chemical reaction of some brain cells. This reaction is new, for the dog had never met this man before. The brain cells are now sensitized, a special mechanism is established which produces the same reaction whenever the same scent is perceived by the dog.

The analogy with adaptive enzymes and antibodies is quite close. We have at the start the untrained cells, potentially capable of being trained; the establishment of the reaction, or the setting up of a special mechanism by the first stimulus; the same reaction always released by the same stimulus; and the absolute specificity of the reaction. It may be added that young animals learn more readily than older ones.

SUMMARY

Adaptability is one of the outstanding properties of organisms. It involves many changes of biochemical reactions, some quantitative, some even qualitative. The author has pointed out that some well-studied qualitative adaptations of biochemical functions show a number of identical characteristics, and therefore might possess the same fundamental mechanism. These adaptible functions are: formation of adaptive enzymes; formation of antibodies; response to organizers; and (perhaps) ability to form associations.

If these functions, or some of them, have a similar chemical foundation, it would be possible to attack the study of this mechanism from different sides. Any advance in the knowledge of this mechanism made with any of these functions is likely to throw new light on all others as well.

REFERENCES

GAY, F. P., 1935. *Agents of Disease and Host Resistance.* Springfield; Thomas p. 333.
HEGARTY, C. P. 1938. Thesis; Cornell University, 1939. *Journ. Bact.* (in press).
KARSTRÖM, H. 1930. Thesis, Helsingfors 1938. *Ergebnisse der Enzymforschung* **7**: 350.

THE GROWTH AND RETROGRESSIVE CHANGES IN CARTILAGE AND BONE OF THE GUINEA PIG PRODUCED BY POTASSIUM IODIDE*

By

MARTIN SILBERBERG AND RUTH SILBERBERG

From the Laboratory of Research Pathology, Oscar Johnson Institute, Washington University School of Medicine, St. Louis

(*Received December 31, 1938*)

In the course of our investigations concerning the effects of various hormones on cartilage and bone of immature guinea pigs it could be shown that feeding of thyroid substance enhances the growth of cartilage, accelerates its differentiation, and causes absorptive processes in the bony substance.[1] Furthermore, Loeb and coworkers have shown that in young guinea pigs, potassium iodide during the first two or three weeks may exert a stimulating effect on the thyroid gland.[2] It seemed, therefore, of interest to compare the effects which these two substances exert on cartilage and bone in the guinea pig.

MATERIAL AND METHODS

In these experiments we used twenty male guinea pigs, weighing between 175 and 195 grams at the beginning of the experiments. These animals also served for certain investigations of Dr. Leo Loeb, to whom we are greatly indebted for permission to make use of this material. The guinea pigs received daily intraperitoneal injections of potassium iodide. Ten animals were given 0.01 gm. potassium iodide dissolved in 1 cc. of 0.9% saline solution daily, while ten others received a daily dose of 0.05 gm. of this salt dissolved in 1 cc. of 0.9% saline solution. After 4, 6, 10, 12, 15 and 20 days, the animals were sacrificed; tibia and ribs were removed for microscopic examination. Our method of fixation, decalcification and staining has been fully described previously.[3]

OBSERVATIONS

After six days the guinea pigs showed only a slight gain in weight. After ten to twelve days the gain in weight was definite, and after fifteen to twenty days the gain averaged about 30%.

Microscopic examination:

1. *Epiphyseal line:* After four days the zone of endochondral ossification was of normal width. Between the sixth and the twelfth days the epiphyseal zone became somewhat wider than it was ordinarily; but at still later stages the width returned again to normal.

Under usual conditions, one row of cartilage consists of about 10 columnar and 4 hypertrophic cells. In the following table the first figure indicates the number of hypertrophic cells, and the second that of the columnar cartilage cells. These

* These investigations were carried out with the aid of grants from the International Cancer Research Foundation and from the Committee on Scientific Research of the American Medical Association.

figures show, therefore, the changes which take place in the number of cells of each kind under the influence of potassium iodide, and they indicate also the ratio between the hypertrophic and columnar cartilage cells. Each of these figures is based upon two individual counts.

TABLE

Dose of KI	After 4 days	6 days	10 days	12 days	15 days	20 days
0.01 gm.	5:12	6:12–13	6:12	6–7:14–15	5:13–14	4:12–13
0.05 gm	6:12	6–7:13	6:13–14	6:14	4–5:12	4:12–13

We may conclude from this table that in the guinea pigs fed 0.01 gm. KI daily the number of columnar as well as of hypertrophic cartilage cells increases. This increase begins as early as four days after the first dose of KI has been given; the number of both kinds of cells continues to increase and reaches a maximum about twelve days after the beginning of the experiment, when it begins to decline; however, as late as after twenty days there is still an increase in the number of columnar cells. In addition, as early as four days after the beginning of the experiment the intercartilaginous matrix appears somewhat swollen and vacuolated. These changes also increase with the progress of the experiment and are still pronounced after fifteen to twenty days.

The conversion of the resting cartilage cells into columnar cartilage is intensified. The nuclei of the former take on a spindle-like shape and stain more darkly with hemotoxylin and the cytoplasm shows a considerable enlargement.

After four days' treatment only a few mitoses are found in the columnar cartilage cells, a considerable increase is noted after six days, and after ten days the mitoses have become very numerous. Following this period a gradual decrease in the mitotic activity is noticeable. However, the time of maximum increase depends also upon the amount of KI which is given; under the influence of the higher dose the maximum effect is reached at an earlier date than after treatment with the smaller dose, and likewise the decline in cells sets in earlier, the endpoint, therefore, being reached correspondingly sooner. Thus, after fifteen days the columnar cartilage of the epiphysis in the animals treated with 0.01 gms. of potassium iodide still shows mitotic proliferation here and there, whereas, in the guinea pigs which had received 0.05 gms., mitoses are scarcely seen at this stage. Likewise, the hyperplasia and hypertrophy of the epiphyseal cartilage are still very marked after fifteen days in the animals which had been given 0.01 gms. of potassium iodide, while in the guinea pigs treated with 0.05 gms. these changes have already begun to recede. After twenty days the number of the hypertrophic cells has again become normal, although, as stated above the number of the columnar cells is still above normal. As far as the degree of intensity of the changes which take place under the influence of this substance is concerned, no marked difference is observed between the effects produced by the smaller and larger doses.

Associated with these growth processes are retrogressive ones. Beginning at ten days and proceeding with increasing intensity up to fifteen days, a disinte-

gration of some columnar cartilage cells occurs; the cell-nuclei become pyknotic; cell debris is frequently found. From this time on these cells gradually disappear; after twenty days disintegrated cells are seen only occasionally, the histological structure of the tissue having become normal again.

2. *Chondrophyte:* The cells situated in the lateral protuberances show a behavior similar to that seen in the epiphyseal line. They are slightly increased in number and the intercartilaginous stroma is somewhat diminished, but in addition there occurs, to a moderate degree, an increased differentiation of the peripheral precartilaginous cells into mature euhyaline cartilage cells and a marked hypertrophy of the fully matured euhyaline cartilage, as is indicated by the appearance of cells with dark bluish-stained larger nuclei and an enlarged cytoplasm. In the most advanced cases a degeneration of such hypertrophic cells may be detected in various places. After fifteen days these slight growth processes recede again and after twenty days a normal condition has been almost fully regained.

3. *Bone marrow:* The breakdown of the hypertrophic cartilage cells by advancing bone marrow and the replacement of the cartilage cells and the ground-substance by bone are interfered with the more, the greater the increase in number of the hypertrophic cartilage cells, the maximum of interference being reached therefore between six to twelve days after the beginning of the KI administration. In the subepiphyseal layer and along the endosteum the lymphoid marrow is partly replaced by a fibrous connective tissue in which a few mitotic figures are seen. Furthermore, instead of the ordinary bony trabeculae, strands of hypertrophic cartilage cells, in which calcium salts are deposited but around which ossification is incomplete or lacking entirely, are seen reaching into the bone marrow. These strands are surrounded by a connective tissue which contains epithelioid osteoblastic cells, but they disappear after fifteen days, when the structure of the bone marrow becomes again fairly normal. The capillaries are congested.

4. *Joint:* The capillaries corrode and finally perforate the bony border lamella between the cartilage and the marrow, and may grow farther into the transitional zone of the cartilage. The hypertrophic and calcified cartilage cells are readily absorbed by chondroclasts. As early as after four days the cartilage cells of the pressure zone, i.e., the innermost zone of the cartilage lining the cavity of the joint, become more numerous and also increase in size. This change is more marked after six days. At the same time the cells of the transitional zone begin to proliferate and take on a perpendicular arrangement, four or more of these cells being surrounded by a single capsule; concurrently, the stroma decreases in amount. After twelve to fifteen days the cells have grown considerably, their outlines have become indistinct, the cytoplasm is partly liquefied, and also the nuclei have become very large. As a result of the liquefaction of the cytoplasm some nuclei seem to be lying in an alveolar space. These changes in the joint develop somewhat later than those in the epiphyseal line, but they also last somewhat longer, and after twenty days, when the conditions in the epiphyseal line have already become again almost normal, there are still lesions recognizable in the joint, although here also they may have decreased to some extent.

5. *Shaft:* The periosteal area is thickened and the zone of periostal cells has been replaced by a dense fibrous tissue, which has reached its maximum size after ten to twelve days and in which some connective tissue cells may be in the process of mitosis. However, mitoses here seem to be less numerous after administration of potassium iodide than after administration of thyroid substance. Likewise, absorption of bone by this parostal tissue is less active than in guinea pigs which have received thyroid hormone. However, even here there is still a great deal of lacunar and osteoclastic absorption of the compact bone-substance present, which leads to the formation of irregular grooves in the latter. After fifteen days the absorptive processes recede, and after twenty days they have become very slight.

6. *Ribs:* The changes are similar to those in the epiphyseal line. From the fourth day on up to the fifteenth day the intercartilaginous chondromucoid ground-substance becomes more and more swollen and vacuolated. As these retrogressive changes advance, the cell nuclei of the resting cartilage may in the end become pyknotic and cell debris may be found in various places. At the same time the bony trabeculae are thinned out and ultimately may become absorbed by osteoclasts; also the compact bone substance of the ribs is somewhat rarefied as a result of lacunar and osteoclastic absorption. This condition is, however, balanced by the apposition of bone by the activity of osteoblasts. The periostal and parostal cells undergo moderate proliferation by way of mitoses; but all these changes are less pronounced than in animals in which thyroid hormone has been administered. The lesions are most marked after twelve days and at that period a slight tendency of the marrow to undergo fibrosis becomes noticeable. After twenty days, almost normal conditions have been restored.

DISCUSSION

In immature guinea pigs intraperitoneal injections of 0.01 and 0.05 gm. of potassium iodide, extending over various periods up to twenty days, influence the growth and differentiation of the euhyaline cartilage of the epiphyses, the ribs and the joints as follows: an increased development of resting into columnar cartilage takes place; quite frequently a slight proliferation of the latter, partly by way of mitosis, occurs and the conversion of the columnar into hypertrophic cartilage is stimulated. In some instances, particularly during the period extending from four to ten days after the beginning of the experiment, the formation of calcified, but not ossified or only incompletely ossified, strands of hypertrophic cartilage indicates a disturbance of endochondral ossification. Associated with these progressive changes are retrogressive alterations in the columnar cartilage cells as well as in the chondromucoid intercartilaginous ground-substance, which latter may undergo swelling, vacuolization, degeneration and disintegration. These changes, as well as the occurrence of mitoses in the columnar cartilage, reach a maximum after ten to fifteen days of treatment; after this period the conditions gradually return to normal, the progressive changes usually receding at an earlier date than the retrogressive ones. In this connection it may be of interest that the above mentioned period of maximum change coincides

with the time of maximum mitotic proliferation of the acinar epithelium of the thyroid gland in guinea pigs receiving intraperitoneal injections of potassium iodide (Rabinovitch[4]). After this maximum growth in cartilage and thyroid has been reached, the proliferative changes gradually recede also in the thyroid gland (Loeb[5]).

Some tendency toward fibrosis is seen in the bone marrow and the periostal connective tissue is likewise increased in amount; furthermore, a slight lacunar and osteoclastic absorption of the compact layer of the bone takes place. The cartilage of the joint undergoes a slight hyperplasia and a distinct hypertrophy; associated with these changes is an accentuated absorption of the bony border lamella and an increased breakdown of the hypertrophic cartilage under the influence of chondroclasts. In advanced cases, circumscribed degenerations of the cartilage of the joint may occur. The alterations in the joint set in somewhat later than those in the epiphyseal line; they are most pronounced after fifteen to twenty days and, correspondingly, are still present at a time when conditions in the epiphyseal line have already receded. The findings in the ribs correspond to those seen in the long bones.

Potassium iodide exerts thus a growth stimulation in bone and cartilage as well as in thyroid gland; it also may stimulate yeast cells (Scharrer and Schwartz,[6] Greaves and Zobell[7]). However, this effect does not extend to all kinds of cells and tissues, inasmuch as administration of potassium iodide in guinea pigs does not lead to a rise in proliferative activity of the epidermis or to an increase in the number of the epidermis cells. On the contrary, multiple and even single doses may cause a lowering in both proliferative activity and number of cells in these tissues (Loeb, Haven, Genther and Friedman[8]).

Summarizing the results of our experiments with potassium iodide, one may state that they bear a certain resemblance to those obtained by us after administration of thyroid substance. However, after injections of potassium iodide, the stimulating effects on the growth of cartilage cells, as well as the retrogressive changes in cartilage and intercartilaginous matrix, are more marked than after feeding thyroid substance, whereas the resorptive processes in the bone are less pronounced in the former than in the latter instance. The differences in the action of potassium iodide and thyroid substance are therefore in degree rather than in kind. The same mechanisms apply in the case of administration of potassium iodide as in the case of feeding of thyroid substance and we may, therefore, refer to our previous discussion.

As far as the retrogressive processes in cartilage cells and intercartilaginous matrix and their relationship to the progressive changes in these tissues are concerned, Silberchatz[9] observed apparently an analogous condition in lymphnodes and thymus of mice after administration of potassium iodide. In the cortex of the thymus and in the lymphatic centers of the lymphnodes he found "innumerable pyknoses which had been preceded by a karyokinetic stimulation of the cells." In this case mitoses precede degenerative changes, whereas in cartilage conditions are reversed.

As our investigations stand at present, no definite statement can be made as

to the mechanism underlying the action of iodine. Further studies are needed to determine whether the effect on cartilage is a direct one or whether it is brought about by the intermediation of the thyroid gland. Furthermore, we must consider the possibility that the action of the thyroid hormone on the skeleton is at least partly due to its iodine content.

SUMMARY

Daily intraperitoneal injections of 0.01 and 0.05 gm. of potassium iodide in immature male guinea pigs for periods up to twenty days have a stimulating effect on the proliferation and differentiation of the euhyaline cartilage of the epiphyses, joints and ribs. At the same time there occur retrogressive processes in cells and intercartilaginous matrix, as well as increased destructive changes in the joints caused by the activity of chondroclasts and a slight absorption of bony substance. These changes reach a maximum after ten to fifteen days and then they gradually recede, until after twenty days an almost normal condition has again been reached. As compared with the action of thyroid substance, potassium iodide produces a more marked growth as well as retrogressive changes in the cartilage, but it is less effective than thyroid substance as far as the absorption of bony substance is concerned.

REFERENCES

1. SILBERBERG, M. and SILBERBERG, R. 1939. The effects of thyroid feeding on growth processes and retrogressive changes in bone and cartilage of the immature guinea pig. GROWTH 2 (4): 327–333.
2. LOEB, L. 1920. The influence of iodine on hypertrophy of the thyroid gland. *Jour. Med. Res.* **41**: 481–494.
   1926. Further investigations of the influence of iodine on hypertrophy of the thyroid gland with an interpretation of the differences in the effects of iodine on the thyroid gland under various pathologic conditions. *Am. Jour. Pathol.* **2**: 19–32.
   GRAY, S. H. and LOEB, L. 1928. The effect of the oral administration of potassium iodide and thyroid substance on the mitotic proliferation and structure of acini in the thyroid gland in guinea pigs. *Am. Jour. Pathol.* **4**: 257–270.
   LOEB, L. 1929. The structural changes which take place in the thyroid gland of guinea pigs during the process of compensatory hypertrophy under the influence of iodine administration. *Endocrinology*, **13**: 49–62.
   MARGOLIN, E. L. 1937. The effect of small doses of potassium iodide on the thyroid gland of the guinea pig. *The Jour. of Pharmacology and Exp. Therapeutics* **57**: 277–283.
3. SILBERBERG, M. and SILBERBERG, R. 1938. Effects of anterior pituitary implants and extracts on epiphyses and joints of immature female guinea pigs. *Arch. of Pathol.* **26**: 1208–1225.
4. RABINOVITCH, J. 1929. The effect of intraperitoneal injection of potassium iodide on the proliferative activity of the thyroid gland in guinea pigs. *Am. Jour. Pathol.* **5**: 91–97.
   1930. Changes in thyroid gland of guinea pigs following a period of administration of potassium iodide. *Am. Jour. Pathol.* **6**: 71–74.
5. LOEB, L. 1929. The influence of variations in size of the remaining part of the gland, in mode of administration and in quantity of potassium iodide on the hypertrophy of the thyroid in the guinea pig. *Am. Jour. Pathol.* **5**: 79–86.
6. SCHARRER, K. and SCHWARTZ, W. 1927. Die Wirkung des Jods auf Hefe. *Biochem. Zeitschr.* **187**: 159–179.
7. GREAVES, J. E., ZOBELL, C. E. and GREAVES, J. D. 1928. The influence of iodine upon the growth and metabolism of yeasts. *Jour. Bact.* **16**: 410–430.
8. LOEB, L., HAVEN, F. L., GENTHER, I. T. and FRIEDMAN, H. 1930. The effect of administration of thyroid substance, of potassium iodide and of extirpation of the greater portion of the thyroid gland on the epidermis of the guinea pig. *Anat. Rec.* **46**: 65–80.
9. SILBERCHATZ, S. 1931. Sur les propriétés caryoclasiques des iodures. *Compt. Rend. Soc. Biol.* **107**: 1570.

d-VALINE IN DEVELOPMENTAL GROWTH

By

FREDERICK S. HAMMETT AND REBECCA COMEE

The Research Institute of The Lankenau Hospital, Philadelphia

Aided by Grants from The International Cancer Research Foundation and the Blanche and Frank Wolf Foundation, Inc.

(*Received for publication January 6, 1939*)

d-Valine is the first of the naturally occurring branched-chain alpha amino acids to be explored with respect to participation in the growth picture of *Obelia geniculata*. Two series of experiments were run with two different sets of workers, one in 1933 and one in 1938. There were 22 experiments in 1933 in concentrations ranging from M/50,000 to M/3400. In 1938 there were 50 experiments in concentrations ranging from M/200,000 to M/12,500. Close to 8000 animals were used in 1933. About 26,000 were used in 1938. The procedure was as usual as described in previous reports. Analysis is made from the 1938 data since these were obtained with a more highly purified d-valine than that of 1933. Further the number of experiments done at any given concentration in 1933 was hardly adequate for evaluation in the case of this particular amino acid. As may be seen, the 1933 data serve in most cases as sustaining evidence for the conclusions drawn from the 1938.

RESULTS

Table 1 suggests that d-valine acts to hold back obelia growth. The evidence is that when all activities are combined test growth tends to be less than control at most every concentration. This would indicate that the compound is highly toxic to obelia. The conclusion is not sustained, however, with respect to obelia growth-as-a-whole when more detailed analysis is made.

In order to provide demonstration data Table 1 has been broken down into its components as in the uracil report (Hammett '36) so that the direction of test deviation from control for each separate growth expression of each separate growth activity is given as in Table 2.

This shows that gonophore development was continuously retarded throughout its course at all concentrations save the M/200,000. Test growth of gonophores was less than control in 25, or 86 percent of the 29 deviations. On the other hand all other test growth was less than control in but 76, or 53 percent of its 143 deviations. This ratio held its order of magnitude at all concentrations save M/25,000. Here there was general retardation of high order. But since it did not persist in the higher concentrations and since the 4 days during which these 10 experiments were run were the hottest of the season, it is presumed that this rather than the amino acid was the major factor. It appears then that a large part of the gross retardation is attributable to a special sensitivity of the gonophores to d-valine.

With these taken out there remains no valid evidence that the compound is toxic to obelia growth-as-a-whole at any of the concentrations used here; save possibly the very highest or M/4000 range.

These being the appearances the remaining analysis is made on the data of hydranths and stolons alone at all concentrations from M/200,000 to M/3400. Unless significant difference makes separation necessary the 1938 and the 1933 data are considered as one set. Those of 1938 are taken as the standard because of greater number of experiments done at a given concentration and because of improved technic in the later year.

The developmental activity of Initiation was essentially unaffected by d-valine. The evidence—from Table 1—is that test was greater than control in 2, less in 2, and equal in 4 of the 8 sets of experiments; that it was greater in 20,

TABLE 1

TREND OF TEST DEVIATION FROM CONTROL WITH RESPECT TO DEVELOPMENTAL AND METABOLIC ACTIVITY IN THE EIGHT *d-VALINE* CULTURES. ALSO THE NUMBER OF COLONIES, HYDRANTHS, AND GONOPHORES AT THE STATED CONCENTRATIONS

	1938												1933*											
M/1000	200			100			50			25			12.5			8.3			6.7			4.0		
# Expt.	10			10			10			10			10			6			6			7		
#	Cont.		Test	Cont.		Test	Cont.		Test	Cont.		Test	Cont.		Test	Cont.		Test	Cont.		Test	Cont.		Test
Cols.	150		150	150		150	150		150	150		150	150		150	75		75	76		76	86		86
Hyd.	2674		2673	2571		2556	2594		2578	2550		2553	2576		2572	1116		1121	1008		1010	1207		1198
Gon.	246		293	289		311	370		335	328		313	302		315	97		97	121		131	131		139
	GROWTH																							
	+	−	=	+	−	=	+	−	=	+	−	=	+	−	=	+	−	=	+	−	=	+	−	=
Init.	2	3	2	3	3	1	4	1	2	1	5	1	0	3	4	2	3	2	3	3	1	5	1	1
Prol.	3	4	1	5	2	1	2	5	1	2	3	2	4	3	1	2	2	4	2	2	3	2	2	4
Diff.	2	5	3	2	5	3	3	2	5	5	4	1	2	6	2	3	3	3	1	3	4	1	5	3
Orgn.	2	1	6	2	3	3	2	4	3	0	6	3	2	2	5	3	2	3	2	2	4	0	2	4
	METABOLISM (By Single Experiment)																							
Main	2	5	3	3	5	2	7	2	1	6	2	2	1	7	2	2	4	0	4	1	1	3	2	2
Catb.	5	2	3	5	4	1	4	5	1	6	2	2	3	7	0	1	2	3	3	3	0	2	4	1
	METABOLISM (By Combined Experiment)																							
	Cont.		Test	Cont.		Test	Cont.		Test	Cont.		Test	Cont.		Test	Cont.		Test	Cont.		Test	Cont.		Test
Main	65.0		62.5	63.9		63.7	66.7		69.0	66.7		67.1	71.0		67.7	75.9		74.7	74.4		78.2	77.7		79.6
Catb.	83.6		85.6	86.5		89.0	84.2		83.0	83.6		85.2	85.8		81.7	94.4		94.8	87.0		87.8	79.7		78.0
	Increase in Percentage Regression																							
	7.1%			0.6%			−6.9%			−1.2%			11.4%			5.0%			−14.8%			−5.8%		

* M/8300 bracket includes one experiment done at M/12,500 and one done at M/10,000.

* M/4000 bracket includes three experiments done at M/5000; two at M/4000 and two at M/3400.

* One experiment done at M/50,000; one at M/25,000; and one at M/16,700 omitted from the 1933 tabulation.

less in 20, and equal in 8 of the 48 expressions given by the 8 sets of experiments; and that it was greater in 2, less in 1, and equal in 3 of the 6 potentialities when each is combined into a single value for the 50 1938 series; while in the 19 recorded for 1933 it was greater in 1, less in none, and equal in 5.

Apparently Anlagen formation or productivity was adversely affected by the amino acid. Test gonophore production was no greater than control in any of the 8 sets but was less in 2, while new hydranth production was less in 6 and greater in but 2 (Table 2).

Proliferation expression also was essentially unaffected by d-valine. Thus test was greater than control in 2, less in 1, and equal in 5 of the 8 sets of experiments; it was greater in 21, less in 15, and equal in 11 of the 47 instances given by the 8 sets of experiments; and it was greater in 1, less in none, and equal in 5 of the 6 potentialities when each is combined into a single value for the 50

TABLE 2

GIVES THE *Direction* OF TEST DEVIATION FROM CONTROL IN EACH OF THE SEPARATE GROWTH EXPRESSIONS ACCORDING TO DEVELOPMENTAL ACTIVITY AT EACH OF THE STATED *d-VALINE* CONCENTRATIONS

M/1000	1938					1933		
	200	100	50	25	12.5	8.3	6.7	4.0
				INITIATION				
Anlagen								
Hydranth	−	−	−	−	−	−	+	+
Gonophore	=	−	=	−	=	=	=	=
Recurrence								
Complete	−	+	=	=	−	−	+	+
Senile	−	+	+	−	=	=	+	+
Past	=	=	+	+	=	+	−	+
Regeneration								
Broken	+	−	+	−	−	+	−	−
Vasculogenesis								
Stolon	+	+	+	−	=	−	−	+
				PROLIFERATION				
Hydranths								
Bud	+	+	+	−	−	=	=	=
Complete	−	+	+	=	−	=	−	=
Senile	−	+	−	+	+	+	0	−
Past	+	+	−	−	+	+	+	+
Broken	+	=	−	=	+	=	+	+
New	−	+	=	−	+	−	−	=
Gonophores								
Bud	=	−	−	+	−	−	=	=
New	−	−	−	−	=	=	=	−
				DIFFERENTIATION				
Hydranths								
Bud	−	−	−	−	=	+	−	−
Half	=	=	=	=	=	=	=	=
Complete	=	+	+	+	−	+	0	0
Senile	−	−	−	−	−	0	0	+
Past	+	+	+	+	+	−	=	=
Broken	−	−	+	+	−	=	=	−
New	−	=	=	+	+	+	−	−
Metaplasia								
Stolon	−	=	=	−	−	−	+	=
Gonophores								
Quarter	+	−	=	+	−	=	=	=
Half	=	−	=	−	−	−	−	−
				ORGANIZATION				
Hydranths								
Bud	−	−	−	−	=	=	=	−
Half	=	=	=	=	=	=	=	=
Three-Quarter	=	=	=	=	=	=	=	=
Senile	=	0	+	−	+	0	0	0
Past	=	−	−	−	−	−	+	=
Broken	+	=	+	−	=	−	−	−
New	+	−	=	−	+	+	+	=
Gonophores								
Half	=	+	−	=	=	=	=	0
Three-Quarter	=	+	−	−	−	=	−	0

1938 experiments; while in the 19 for 1933 it was greater in 2, less in 1, and equal in 3.

Differentiation, on the other hand, showed a slight trend to retardation as a developmental activity. Thus test was less than control in 3 and greater in none of the 8 sets of experiments; it was less in 25 and greater in but 17 of the 64 expressions given by the 8 sets of experiments; and in both the 1938 and 1933

series test was less in 3 and greater in 2 of the 8 potentialities when each is combined into a single value for the 50 and 19 experiments respectively.

Organization also may have been held back a trifle; but the reaction was more a hint than a fact. Thus test was less than control in 3 and greater in 2 of the 8 sets of experiments; it was less in 17 and greater in but 9 of the 52 expressions afforded by the 8 sets; and it was less in 2 and greater in 1 of the 7 potentialities when each is combined into a single value for the 50 experiments of 1938; while for the 19 recorded of 1933 it was less in 1 and greater in 1.

Neither Maintenance nor Catabolism expression showed any consistent reaction to d-valine.

DISCUSSION

The passivity of living organisms to valine is a matter of record. While Ort and Bollman ('27) found some stimulating effect on metabolism and Kisch ('32) got increase in respiration in one out of three experiments, Melon ('27) obtained no effect on perfusates of isolated organs, and Terroine, Trautmann, Bonnet, and Jaquot's ('24) results were inconclusive. All these data from related observations coincide with ours that the compound has an indeterminate effect on maintenance and regression.

The Obelia data therefore give no evidence of specific dynamic effect, a result which is again consistent with the direct measurements of Rapport and Beard ('27) who find this amino acid lacking in this property.

In the growth field the observations are similarly unenthusiastic. Burrows and Neymann ('17) got only inhibition and toxicity; Gordon and M'Leod ('26) report no effect on bacterial growth; Holt ('28) could find no effect on production of giant yeast cells; Odiette and Truhaut ('33) wrote it had no action on tissue cultures.

Yet the compound is present in high amount—if the analyses are reliable—in such vitally important proteins as casein, lactalbumen, ovalbumen, and gliadin (Mitchell and Hamilton '29). It is not there for the fun of it.

If—as it is beginning to seem possible from these studies—the growth significant factors reside in special chemical groupings which exist or are liberated by intermediary metabolism, then some light may be thrown on the problem by the recent work of Corley and collaborators ('37:'38). From them comes the report that d-valine is not easily deaminated in the dog, and that the presence of a CH_3 group on the NH_2-bearing carbon makes N-removal difficult, while one on the contiguous carbon may seriously interfere. It is conceivable that such difficulties may mask any specific growth participation which the compound may have in experiments of the usual type. If this is so experiments must be designed to expose wherein this refractory compound is essential. It is not a common constituent of proteins for nothing.

The Obelia results are by no means insignificant in this matter. They show a definite growth reaction to this amino acid. They do not show that any developmental activity is affected; but they do show that a very definite kind of growth is influenced. For gonophore development is a definitely specific growth activity

of Obelia. Through it and no other are produced the gamete-bearing medusae. Where or at what stage d-valine is a participant in the complex series of chemical process which bring a bud from an anlagen through its expansion into the medusa-containing gonotheca up to the point where these little animals claw their way out into freedom as full-fledged individuals is not surely told by these observations. But very pertinent suggestion is had from the fact that hydranth production from anlagen is seriously affected in the same direction. Now anlagen activity for medusa production in the gonophores must be considerable. And it is interesting to note that the stages whereat this might be supposed to be at a maximum are just those stages whereat the d-valine effect is most pronounced. The correlation would seem to justify the tentative postulate that a site of action of d-valine on gonophore development is in some process concerned with medusa anlagen productivity. Indeed it is possible that it is not gonophore development as such at all which is affected, but more explicitly, anlagen productivity, and that it is the disturbance in this and nothing else which is responsible for the general developmental retardation of the gonophores. To one familiar with this animal it is obvious that a halting in medusa production could well act to hold back gonophore growth as a whole; while the cutting down of new hydranth production from anlagen would have no noticeable effect on development of hydranths already initiated or present.

If the above interpretation be valid, and the data seem to point that way consistently, maybe it is something more than coincidence that d-valine is at one and the same time a generous component of proteins eminently suitable for infantile growth, and a factor of specific influence where such is analogously taking place.

The specificity lies in the fact that so far no other amino acid has shown a like clean-cut effect.

That the influence is retarding rather than forwarding is of no immediate concern. Other amino acids have induced a like direction of reaction (Hammett and Elliott '35:Hammett '37).

SUMMARY AND CONCLUSION

Seventy-five experiments with more than 34,000 animals half of which were exposed to d-valine in concentrations ranging from M/200,000 to M/3400, in two seasons, with two different sets of workers show that the compound,—

1. Has no sure effect on the developmental activities of Initiation, Proliferation, Differentiation, or Organization of colonial-living *Obelia geniculata*.
2. Has no consistent effect on Maintenance, Regression, or Catabolism and hence no Specific Dynamic Effect.
3. Has no evident toxicity.
4. Has a retarding effect on gonophore growth as a whole.
5. Has a localized retarding effect on anlagen productivity.

The postulate is tentatively advanced that d-valine is a participant in some process specifically concerned in anlagen productivity. The gonophore reaction is explainable on the basis of interference with medusa anlagen productivity.

The work reported here was done at the Marine Experimental Station of The Lankenau Hospital Research Institute, North Truro, Massachusetts, with the technical assistance of Misses Chatalbash, Elliott, Padis, Steele, and Hyde and Messrs Toman and Rivard.

REFERENCES

BURROWS, M. T. and C. A. NEYMANN. 1917. Studies on the metabolism of cells in vitro. I. The toxicity of alpha-amino acids for embryonic chicken cells. *Journ. Exptl. Med.* **25**: 93–108.

FILDES, P., and G. M. RICHARDSON. 1935. The amino acids necessary for the growth of Cl. sporogenes. *Brit. Journ. Exptl. Path.* **16**: 326–335.

GORDON, J., and J. W. M'LEOD. 1926. The inhibition of bacterial growth by some amino acids and its bearing on the use of tryptic digests as culture media. *Journ. Path. Bact.* **29**: 13–25.

HAMMETT, F. S., and M. L. ELLIOTT. 1935. Developmental growth and the amino acids. II. l-tryptophane. *Protoplasma 23* **4**: 457–465.

HAMMETT, F. S. 1936. Developmental growth and nucleic acid components. I. *Uracil. Biodynamica, No. 9*, March 1–12.

HAMMETT, F. S. 1937. d-Lysine and growth. This Journal, **1**: 187–193.

HOLT, M. 1928. Giant yeast cells. *Trans. Roy. Soc. Canada* **(3)22**: 267–270.

KISCH, B. 1932. Further studies on tumor respiration. I. Amino acids. *Biochem. Z.* **247**: 354–364:459–463.

LEIGHTY, J. A. and R. C. CORLEY, 1937. Amino Acid Catabolism. IV. The fate of certain alpha-amino acids administered by subcutaneous injection to the normal dog. *Journ. Biol. Chem.* **120**: 331–334.

MELON, L. 1927. The action of amino acids on the metabolism of isolated organs. *Arch. Intern. Physiol.* **28**: 29–57.

MITCHELL, H. H. and T. S. HAMILTON. 1929. *The Chemistry of Amino Acids*. Chem. Catalog. Co., New York.

ODIETTE, D. and R. TRUHAUT. 1933. Rapports entre l'action des acides amines sur la croissance des cellules *in vitro* et leur pouvoir mercuro-reducteur. *Compt. rend. Soc. Biol.* **113**: 1484–1486.

ORT, J. M. and J. L. BOLLMAN. 1927. Catalytic and specific dynamic actions of certain amino acids. *Journ. Am. Chem. Soc.* **49**: 805–810.

RAPPORT, D. and H. H. BEARD. 1927. The effects of protein-split products upon metabolism. II. The individual amino acids of fraction 1 of the butyl alcohol extraction, and their relation to the specific dynamic action of protein. *Journ. Biol. Chem.* **73**: 299–319.

SNYDER, H. and R. C. CORLEY. 1938. Amino acid catabolism. V. The influence of structural configuration on the deamination of alpha-amino acids in the normal dog. *Journ. Biol. Chem.* **122**: 491–498.

TERROINE, E. F., S. TRAUTMANN, R. BONNET and R. JAQUOT. 1924. Cultivation of molds on amino acids and mechanism of specific dynamic action. *Compt. rend. Soc. Biol.* **178**: 1488–1491.

STUDIES OF UNFERTILIZED HUMAN TUBAL OVA NO. 2: ANATOMIC OBSERVATIONS OF THREE WITH REMARKS ON OVULATORY TIME*

By

BERNARD J. MILLER, GILSON C. ENGEL AND STANLEY P. REIMANN

From The Lankenau Hospital Research Institute, Philadelphia

(*Received December 31, 1938*)

The work here reported was originally undertaken with the view of determining the possibility of parthenogenetic activity in the unfertilized human tubal ovum.† During the course of the experiment facts of varying nature were encountered with relation to phases other than that of parthenogenesis that we feel should be reported at this time.

Five unfertilized human tubal ova have been recovered, three of which will be reported at this time. Information concerning the remaining two will be withheld temporarily until certain points are clarified from future ova.

The ovary is composed of cortex and medulla. The cortex is covered by an outer connective tissue layer, the tunica albuginea which gradually blends into the broader cortex proper in which are enclosed the primordial ovarian follicles. The primordial follicles are lined with a thin layer of epithelial cells. A connective tissue capsule, the theca folliculi, encloses the primitive ovarian follicle. The medulla is the supporting structure for the cortex and consists of spindle-shaped cells, connective tissue, an abundant supply of blood vessels and nerves with some smooth muscle fibers.

The oocyte in the primordial follicle is stimulated to mature apparently by the anterior pituitary gonadotropic hormone. As the follicle increases in size it recedes from the surface toward the more vascular medulla. During this period the epithelial layer of the follicle becomes cuboidal, several layers thicker and is now termed the membrana granulosa. The follicle becomes larger due to the formation of an albuminous fluid termed the liquor folliculi. At one pole of the follicle the granulosa cells become collected into a small mass termed the cumulus oophorus. In this "hill" the ovum is embedded. The ovum increases in size and yolk granules appear in the cytoplasm. Around the periphery of the ovum, a thin transparent capsule forms, the zona pellucida. On close observation the capsule appears striated, forming the zona striata of the ovum.

The theca folliculi cells change their morphology from connective tissue-like to cuboidal and gland-like containing yellowish lutein granules. Maturation starts in the cortex but as the graafian follicle matures the theca works toward the surface and ruptures. The matured ovum is thus extruded from its follicle into the peritoneal cavity as the process of ovulation.

Details of the time element in this maturation process in the human female are still in dispute. Allen, Bland, et al., (1) by a study of five human tubal ova

* Aided by a grant from Mrs. L. Elizabeth Nax.

† To be published in the Archives of Pathology.

recovered on the fourteenth, fifteenth, and sixteenth days and an examination of the follicles from which they were extruded, set the period of ovulation as between the eleventh and the thirteenth day after the onset of the previous menses. Burr, (2), by a potentiometric device, recorded a sharp increase in potential in the human female between the ninth and eleventh days, and also on the fifteenth day, before the onset of the next menses, presumably making the period of ovulation as the thirteenth day after the onset of the previous menses. The best chance of obtaining freshly discharged ova seems to be within this period.

On the basis of these considerations we planned to recover unfertilized human ova at different stages.

The method is simple from the clinical standpoint. A patient with fibroleiomyomata requiring hysterectomy is carefully questioned as to her cycle. Admission to the hospital for operation is planned on the thirteenth or fourteenth days before the onset of the next menses in a twenty-eight day cycle case, or the twelfth day before in a twenty-six day cycle case. In other words, one day less the half way in the cycle before the onset of the next menstruation. The patient is operated on the following day.

Spinal anesthesia was used so that the damage theoretically possible to the ovum from general anesthesia was avoided. A paramedian incision was made and the bowel packed away from the pelvis with gauze pads. Before any manipulation was done the right tube was picked up with a Babcock forceps so as not to damage the tube or its contents. A circular ligature of plain catgut was placed around the fimbriated end of the tube. The hysterectomy was then performed in the usual manner. Both tubes were removed "en bloc" with the uterus, being extremely careful not to traumatize the tubes. As soon as the tubes and uterus were removed they were taken to the laboratory.

The tubes were then removed from the uterus at their proximal ends by cutting deep into the wall of the uterus at the point of junction, in such a manner that the greatest length of the tube was preserved intact. To prevent buckling of the tube in the subsequent manipulation, the mesosalpinx was dissected away from the tube. A #20 Leur type needle was then inserted into the fimbriated end. This operation must be carefully performed since the needle might be forced far into the lumen in which case the ovum, if present, would not be washed down. The needle may then be tied in place, or else held firmly between the thumb and index finger of the left hand. The syringe is then manipulated with the right hand and ten to twenty cubic centimeters of Locke's solution forced through the tube. Recently we have been using a mixture of blood serum and Locke's solution as a washing medium.* The washings are collected in 5 cm. diameter, sterilized watch glasses. Within five or ten minutes the ova rapidly settle to the deepest portion of the watch glass where they may be located at a magnification of about 100. Photomicrographs of the ova were taken at any stage which seemed interesting by a Leica microcamera attachment or a 5″ by 7″ Zeiss plate camera. In some cases the ova were observed

* Personal communication of Dr. Gregory Pincus.

over a period of time in the original wash fluid. In other cases the ova were transferred to a hanging drop of human blood serum to which had been added a droplet of ethyl ester of acetic acid, and then were placed upon the warm stage of the microdissecting apparatus.

FIRST SPECIMEN

The first specimen was obtained June 17, 1938, from a white woman, forty-five years of age. Her menstrual periods had been regular, but during the past ten to twelve months had noticed a premenstrual spotting for five to seven days previous to the onset of her normal flow which lasted for two to three days. This patient stopped menstruating June 5; her period lasted three days, making June 17 the fifteenth day after the onset of her previous menses.

The specimen obtained at operation consisted of a fibroleimyomatous uterus, two fallopian tubes, and two ovaries. Both tubes were slightly tortuous and congested. The ovaries were almost equal in size. There was a follicular cyst in one ovary, while the other ovary showed a fresh corpus hemorrhagicum about 1.8 cm. in diameter, which on section proved to be a very young corpus hemorrhagicum in which no lutein cells had as yet been differentiated.

The ovum was identified in the first few cubic centimeters of wash fluid with the 16 mm. objective. It was the most regular of those obtained being an almost spherical mass which rolled over quite easily when agitated. Its diameter, including the zona pellucida, was 81.84 microns. The zona pellucida was clear and distinct and measured between 9 and 11 microns in thickness. There were no cumulus oophorus cells about the ovum and judging from this the age of this specimen was about two days. It was in the tube approximately one day since no cumulus oophorus cells were found.

There were two chromophoric bodies in close proximity to each other directly beneath the zona pellucida. We believe these to be polar bodies since at greater magnification each showed a thickened central portion and an indentation in the center of these areas.

The oocyte did not completely fill the space outlined by the zona pellucida. Although the vitellus was attached to the inner surface of the zona pellucida along most of its surface of contact, it was unattached along a small border giving rise to a pervitelline space on that side. This space was very small and could be seen only by careful variation in focusing. Fig. 1 a.

The cytoplasm of the egg was very granular. The granules were uniformly arranged and varied slightly in their size and degree of coarseness. They appeared to vary between two and four micra in diameter and were of a distinct yellow color.

Within a period of a few hours a vacuole was observed to appear in the cytoplasm. The vacuole migrated to the periphery and then into a pseudopod which soon disappeared. We believe that this was caused by the unadjusted Locke's solution used as a medium (Pincus). Within the next hour the vitellus decreased considerably in size and receded within the zona pellucida causing a great increase in the perivitelline space. Fig. 1 b. We believe that this

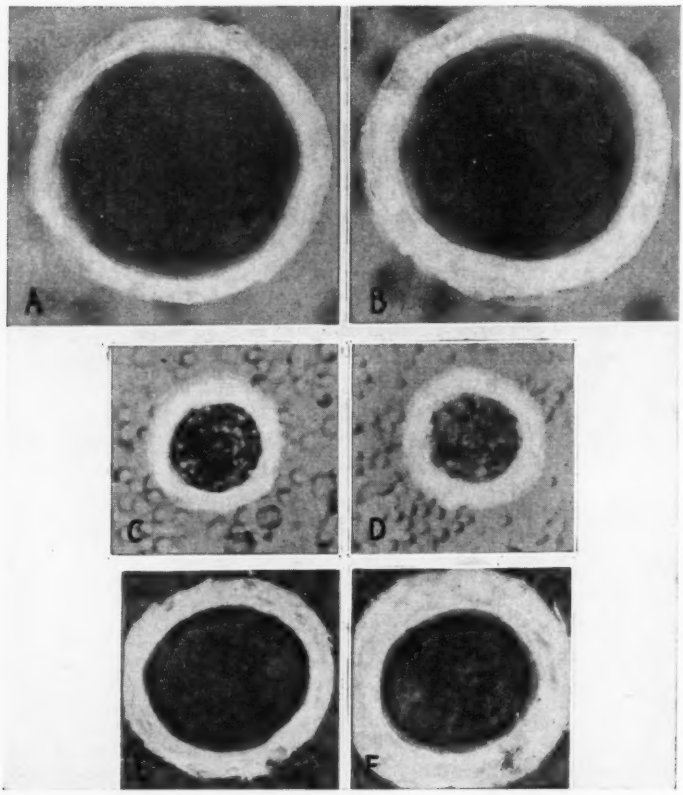


FIG. 1

a. Half hour in Locke's Solution.
b. Four hours in Locke's Solution.
4 mm. objective, 10× periplan eyepiece. Photograph enlarged eleven times.

c. High focus—before transfer to micromanipulator.
d. Low focus—before transfer to micromanipulator.
16 mm. objective, 10× periplan eyepiece. Photograph enlarged eleven times.

e. High focus—before transfer to micromanipulator.
f. Low focus—before transfer to micromanipulator.
4 mm. objective, 10× periplan eyepiece. Photograph enlarged eight times.

A Leica microcamera was used for all photographs.

early shrinkage was likewise due to the unadjusted Locke's solution. Apparently Locke's solution is not isotonic for the human tubal ovum. The portion of the zona that was not visible in the fresh egg had now become visible and quite clear.

SECOND SPECIMEN

The second human specimen was obtained June 24, 1938, from a thirty-seven year old white woman. Four months before, this patient had profuse vaginal bleeding which lasted for ten days. This condition did not return but she was left with abdominal pain. Her menstrual periods had been regular every twenty-eight days and she noticed nothing unusual about the flow. There were no other intermenstrual discharges. Her last period began on June 8, 1938, making June 24 her sixteenth day.

The ovary removed at operation showed a follicular cyst and a ruptured corpus hemorrhagicum which on microscopic examination showed lutein cells which had already been differentiated. Sections of the endometrium showed the glands to be tortuous and rather far apart.

The ovum was found with the 16 mm. objective and measured 70.98 micra in diameter. It was spherical and showed very little variation in mean diameter. There was a distinct limiting membrane, and the indications of a very thin zona pellucida which measured no more than five micra in thickness. In general appearance the ovum was not as "normal" as the first specimen since its cytoplasm was not as uniformly granular nor the zona as thick. Possibly the cause for the variation in morphology was the fact that this ovum was one day older, assuming of course, that both were ovulated at the same time.

At low focus the enclosed vitellus appeared in contact with the zona pellucida at all points. There was no perivitelline space. At very high focus a peculiar vesicular body, triangular in shape, came into view. The body appeared to be peripherally bordered by yolk granules varying in size and shape, but the central portion was quite clear and free of any granular bodies as though it were just an empty space. Fig. 1 c. At slightly lower focus it was totally obliterated and a mass of yolk substance came into view as the floor of the vacuole. **Fig. 1 d.**

The cytoplasm of the ovum was irregular in appearance. A few of the granules were more conspicuous when viewed with a dark blue filter. At low focus many of the large granules and aggregations of the smaller ones came into view. These larger bodies were about ten times the volume of the smallest granules and were of a deep yellow color. The smaller granules appeared more translucent and were of a less intense yellow color. At high focus the vesicular body showed peripheral aggregations of the smaller yolk granules.

THIRD SPECIMEN

The third specimen was obtained July 18, 1938, from a colored woman whose periods averaged twenty-eight day intervals, the flow lasted usually five days and was profuse. On the fifteenth day after the onset of her last menses, the specimen removed at operation consisted of two fallopian tubes, a fibroleiomatous uterus, and one ovary. The ovary was soft, pinkish gray in color and had been partially resected at a previous operation. Sections of the endometrium showed it to be definitely not in the lutein stage, while sections of the corpus hemorrhagicum showed a considerable amount of blood and few lutein cells differentiated.

The ovum was identified under low power and transferred to a hanging drop of human blood serum to which had been added a droplet of ethyl ester of acetic acid. The preparation was then placed in a warm, moist chamber.

The ovum on first appearance was rather large and quite uniform in appearance, measuring 89.64 micra. The oocyte was expanded against the internal surface of the zona pellucida. Fig. 1 e. There was therefore no perivitelline space. The zona pellucida was clear and uniform appearing as a shell totally investing the contained vitellus. At one point on the peripheral margin of the vitellus there was a marginal darkness directly beneath the zona pellucida which appeared to be a polar body. This body became more evident when the ovum was rolled over on the opposite side. By focusing at different levels a darker central portion was seen. There were no vacuoles and as far as observation could tell the ovum appeared fresh. The cytoplasm was not uniform in composition, but yet more homogeneous than the second specimen, and thus was slightly older than other specimens. The various sized yolk granules were seen and variations in the color of these granules were also observed.

The cytoplasm of the ovum was predominantly composed of small yolk granules. These granules were quite translucent to light and were tinged with a faint yellow color. The larger granules were more opaque to light and were more deeply colored. Fig. 1 f. One large chromophoric body was observed near the margin of the ovum. This body appeared to have radiating strands directed toward the zona pellucida and toward the central portion of the ovum. The dark yellow granules were four in number and were evenly distributed throughout the ovum.

DISCUSSION

Twenty-four operative specimens removed during various days of the menstrual cycle have been examined for ova. Five of these, removed on the fifteenth and the sixteenth days yielded ova, while one very large and distorted specimen was removed on the twenty-first day and yielded an ovum with a considerable amount of protein clinging to its surface.

Judging from the experience with these twenty-four specimens, excluding the twenty-one day ovum, the periods of the cycle during which the best chances are present of finding an ovum in the tube are between the fourteenth and the sixteenth day after the onset of the previous menses. The ovum recovered on the twenty-first day was degenerated and apparently had been withheld in the tube since the tube was tortuous and offered resistance to the passage of the wash fluid. We believe the ova entered the tube on the fourteenth day after the last menses assuming that ovulation took place at the same time in all three individuals. This is the obvious conclusion for two reasons. Specimens removed on the thirteenth day failed to yield ova. The specimen recovered on the fifteenth day appeared more homogeneous than the other ova and was devoid of any cumulus cells. Since eggs in the fimbriated and distal portions of the tube are surrounded by cumulus oophorus cells, this fifteen-day egg must have entered the tube the day before, making the fourteenth day the early limit.

The sixteenth day is probably the last day that the egg remains in the tube.

Two specimens removed during the seventeenth day yielded no ova on careful examination although there were ruptured follicles in the ovaries. Again assuming that all ova were ovulated at the same time, and judging from the specimen removed on the sixteenth day, ova in this stage have already begun to degenerate and are about ready to pass from the fallopian tube.

From the microscopic appearance of the endometrium and the follicles from which these ova were ovulated, the indications are that in a normal twenty-eight day cycle the ova definitely enter the fimbriated portion of the tube on the fourteenth day after the onset of the last menses and are not to be found in the tube past the sixteenth day. It also appears that these three ova were all ovulated at approximately the same time in the cycle. Apparently ovulation occurred on the thirteenth day since the corpus hemorrhagicum from which our first specimen (fifteenth day) was ovulated was very young and showed no lutein cells. The follicles from which our second and third ova were ovulated definitely substantiate our deduction that the third specimen was approximately one day older than our first specimen since the lutein cells had just begun to form in the follicle from which our third specimen was ovulated. The second ovum is definitely older than the first or third specimen since the follicle from which this ovum was ovulated showed the largest number of lutein cells already differentiated.

Of all the specimens examined during the course of this study, those yielding ova always showed a definite redness of the fimbriated end of that tube that had previously received the ovulated ovum.

Hypothetically the period that the ovum remains potent in the tube is probably of very short duration, lasting no more than two days. Once past this limit the ovum probably cannot become fertilized. The reasons for this hypothesis are as follows. The first ovum was recovered on the fifteenth day and, as previously stated, was perfectly homogeneous with no signs of disintegration. The second ovum was recovered on the sixteenth day and the cytoplasm had already become vacuolated, and the zona pellucida had already decreased considerably in size. Judging from our finding that the period during which the ovum is in the tube is between the fourteenth and the sixteenth days of a twenty-eight day cycle this ovum was about ready to pass from the tube. Another ovum recovered during the fifteenth day showed cytoplasm slightly granular but not vacuolated, and therefore was probably older than the first fifteen-day specimen. This ovum is the one which became parthogenetically active, whereas efforts to elicit similar response in the sixteen-day ovum were unsuccessful.* Possible the latter was no longer potent and disintegration begins about this time.

None of these ova were fixed, imbedded, sectioned and stained, for the obvious reason that they could not be observed until disintegration took place and also be prepared for sectioning.

SUMMARY

1. Descriptions of three unfertilized human tubal ova are given.
2. Technique of recovery of tubal ova is discussed.

* In press.

3. Judging from the days during which these and other ova were recovered, the ovulatory period is between the eleventh and the thirteenth day after the onset of the previous menses in twenty-eight day cycle cases.

4. Probably the earliest day that the ovum can be found in the fallopian tubes is the fourteenth day after the onset of the last menses.

5. The period that the ovum can be fertilized is probably very short since our first fifteenth-day ovum appeared as the most normal.

6. Ova recovered on the fifteenth day after the onset of the last menses must have been in the tubes at least one day since no cumulus cells were present.

7. Locke's solution when used alone is not isotonic for the human tubal ovum. (Pincus).

BIBLIOGRAPHY

1. ALLEN, E., PRATT, J. P., *et al.* Human Tubal Ova; related early corpora Lutea and uterine tubes. *Carnegie Contrib. to Embryol. No.* **414. 22**, 45–76, 1930.
2. BURR, H. S., MUSSELMAN, L. K., *et al.* Bioelectric Correlates of Human Ovulation. *Yale J. Biol. and Med.*, 1937, **10**, 155.

GUANINE IN DEVELOPMENTAL GROWTH

By

FREDERICK S. HAMMETT

The Lankenau Hospital Research Institute, Philadelphia

(*Received for publication January 23, 1939*)

INTRODUCTION

Guanine is an integral constituent of nucleic acids and hence of nucleoproteins (Levene and Bass 1931). It is therefore present in every nucleus of every living cell. As isolated therefrom it is a compound of singular and characterizing structure, having an amino group on position 2, and an oxygen on position 6 of the pyrimidine nucleus. Because it is present in every cell and because it is individual in composition the conclusion is inescapable that it must be a compound of definite and specific function. This function has not yet been determined. Indeed it has not even been sought for perhaps.

Some have studied how guanine is formed (Mendel and Saiki 1908) and destroyed (Kiesel 1911; Allen and Cerecedo 1933): some have studied its occurrence (Saiki 1910; Wells and Corper 1910): some have studied its liberation (Wells 1912: 1913): and some have supposedly studied its effect on growth (Coppin 1913; Guha and Chakravorty 1934). But nobody has uncovered or even suggested its function. Yet guanine has been known for nearly a century and its structure for close to 50 years.

The same is true in large part of the other integral components of the nucleic acids. The apathy of interest in the functions subserved by these ubiquitous naturally occurring components of living matter is almost incomprehensible. For surely the specific functions of the substances of living are primary: quite aside from their general properties as structural units; as energy producing combustibles; or as contributors to the reaction basis of living.

The results reported here were gotten in an attempt to evaluate the part played by guanine in growth. They are clean-cut and definite. They show that the compound participates in growth as a determinant thereof; that it is something other than a structural unit, a combustible, or a component of the reaction basis for living. Whether or not this is to be taken as expressive of a function is a matter for the future to decide.

PROCEDURE

The guanine used in these experiments was specially purified to physical and chemical constancy in our own laboratory. The compound is quite insoluble; 0.039 gram per 1000 cc. H_2O at 40.1 C°. (Levene and Bass 1931.) The solubility of guanine in sea-water at any temperature is unknown. But it is still very low. Because of this fact it was necessary to use a saturated solution as stock and filtered aliquots therefrom for the cultures. The amount of guanine in these

Aided by grants from the International Cancer Research Foundation, the Blanche and Frank Wolf Foundation, Inc. and the women's auxiliary of the Lankenau Hospital Research Institute for the Promotion of Cancer Research.

aliquots is unknown. And any guess as to the amount presented to the obelia would be misinformative. This ignorance does not prevent elucidation of what guanine does to obelia growth. It merely blocks knowledge of the effective concentration.

The stock solutions were made by triturating and shaking 0.075 gram guanine with 1000 cc. freshly collected sea-water. There was a large residue. This was filtered off. Aliquots of the filtrate were diluted with sea-water to make the test

TABLE I

TEST DEVIATION FROM CONTROL WITH RESPECT TO DEVELOPMENTAL AND METABOLIC ACTIVITY IN GUANINE CULTURES. ALSO THE NUMBER OF EXPERIMENTS, CULTURES, AND GONOPHORES AT THE STATED CONCENTRATIONS

Cc. Stock per 500*	1.5			3.2			6.25			12.5			25.0		
No. Experiments	14			15			14			15			15		
	Cont.		Test	Cont.		Test	Cont.		Test	Cont.		Test	Cont.		Test
No. Colonies	210		210	222		222	210		210	222		222	223		223
No. Hydranths	3696		3705	3999		4008	3663		3663	3946		3926	3863		3870
No. Gonophores	251		263	424		358	431		439	540		502	572		527
							GROWTH								
	+	−	=	+	−	=	+	−	=	+	−	=	+	−	=
Initiation	4	0	3	4	3	0	1	4	2	3	2	2	1	5	1
Proliferation	6	1	1	2	2	4	1	5	2	2	4	2	2	3	3
Differentiation	4	4	2	3	3	4	3	5	2	3	5	2	3	6	1
Organization	5	1	2	3	3	3	5	1	3	4	2	3	2	4	3
							METABOLISM (By Single Experiment)								
Maintenance	9	3	2	6	6	3	4	10	0	6	5	4	5	10	0
Catabolism	6	5	3	7	6	2	8	5	1	10	4	1	6	9	0
							METABOLISM (By Combined Experiment)								
	Cont.		Test	Cont.		Test	Cont.		Test	Cont.		Test	Cont.		Test
Regression %	25.2		23.9	22.2		22.1	25.6		27.2	28.4		29.2	26.1		27.7
Catabolism %	77.2		79.2	84.9		84.6	86.1		87.1	83.5		87.3	84.4		85.8
							Increase in Percentage Regression								
	−5.2			−0.5			6.2			2.8			6.1		

* Stock solution sea water saturated with guanine. Cf. Text.

culture solutions. The amount of filtrate taken to 500 cc. sea-water is shown in Table 1. Otherwise procedure was quite the same as that in all previous reports.

RESULTS

Guanine was toxic to obelia at 6.25/500 cc. concentration and upwards. The evidence is that 15 of the 34 growth expressions were less in test than in control material and but 10 were greater; while in the lower 3.2/500 cc. level test growth was greater in 12 and less in 10. Further—at 6.25/500 cc. test growth was less than control in 3 of the 4 developmental activities, while at 3.2/500 cc. it was less in none. The non-toxic 1.5/500 cc. to 3.2/500 cc. range will therefore be used for decision as to the part played by guanine in growth.

Initiation was forwarded in the presence of guanine. Thus—new bud production in the tests was greater than control in 8 and less in but 3 of the 14 expressions given by the 2 sets of experiments; by concentration it tended to be greater in both series; and when the data are combined—test is greater than control in 4 and less in none of the 7 potentialities for activity expression. New bud production by Recurrence tended to be somewhat better than by other processes. Here

test was superior in 80 percent of the deviations from control while in the other cases it was superior in 67. Stolon production—or vasculogenesis—tended to be specially favored for despite toxicity it was increased in 3 and retarded in none of the 6 sets of experiments.

Test proliferation expression leaned towards enhancement. Thus—it was greater than control in 8 and less in but 4 of the 16 expressions given by the 2 sets of experiments; by concentrations it was greater in 1 and equal in 1 of the 2 series; and when the data are combined—test is greater in 4 and less in but 1 of the 8 potentialities for activity expression. The structures produced by Recurrence were again somewhat more favored than those coming from other sources. Here test was superior in 80 percent of the deviations from control while in the other cases it was superior in but 57.

Test differentiation was not particularly affected by guanine. Thus—it was greater than control in 7 and less in 7 of the 20 expressions given by the 2 sets of experiments; by concentrations test was the same as control in both; and when the data are combined test is greater in 5 and less in 3 of the 10 potentialities for activity expression. The organisms derived by Recurrence seem to maintain their exhibition of favored reaction. Here test was superior in 60 percent of the deviations from control, while in all other cases it was superior in but 50 percent.

Organization was enhanced. Thus—in the tests it was greater in 8 and less in but 4 of the 17 expressions given by the 2 sets of experiments; by concentrations it was greater in 1 and equal in 1; and when the data are combined it is greater in 4 and less in but 1 of the 9 potentialities for activity expression. What is more this betterment extended into the toxic 6.25/500 cc.–12.5/500 cc. zone; a reaction which was not exhibited by any of the other major developmental activities. Here test was greater than control in 9 and less in but 3 of the 18 expressions given by the 2 sets of experiments; by concentrations it was greater in both series; and when the data are combined it is greater in 3 and less in 1 of the 9 potentialities. It is only in the completely toxic 25.0/500 cc. series that organization fails to exhibit enhancement. Organisms coming by Recurrence did not here contribute anything particular. The enhancement was general with possibly the gonophores being somewhat more consistently favored than the hydranths. Thus in the case of the gonophores test was superior in 88 percent of the deviations from control while in the case of the hydranths it was superior in 65.

Maintenance tended to be sustained and regression held back in the non-toxic range. In the higher concentrations these relations were reversed as is usual when no specific dynamic effect intrudes to complicate matters. There was no evidence of specific dynamic action.

Catabolism leaned towards increase in the presence of guanine but the reaction was not severe.

DISCUSSION

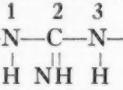
That guanine in non-toxic concentration tends to sustain maintenance is further support for the interpretation from previous results that the imidazole

group is a factor of importance in this reaction (Hammett 1938). It suggests that guanine breaks down into allantoin rather than into urea and a pyrimidine (Hammett and Porter 1938). Consistent with this is the fact that guanine produces like allantoin a generalized tendency to better-than-usual growth and an enhancement of new stolon formation from the cut end of the colonial hydrocaulus (Hammett and Elliott 1936). This interpretation is supported by the finding of Allen and Cerecedo (1933) that guanine goes to allantoin and urea in the dog.

Guanine has no amino group on position 6. It does not—like cytosine and adenine which do have such a group—act to inhibit new growth production by initiation (Hammett, Lavine and Lavine 1937; Hammett 1938). This is indirect support for the concept that amino nitrogen in this place is an inhibitor of initiation (Hammett and Porter 1938).

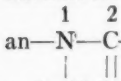
Guanine does have an oxygen attached to position 6 and like those nucleic acid components of similar constitution it acts to forward recurrent growth initiation. Thus is sustained the interpretation that position 6 of the pyrimidine nucleus is of signal importance to the developmental activity of Initiation (Hammett and Porter 1938).

Initiation as a whole is forwarded by guanine. The same is true of arginine (Hammett and Chatalbash, 1935). Although uracil and hypoxanthine have similar effects the reaction is not complete as in the case of guanine and arginine. Regeneration is held back by uracil and stolon production by hypoxanthine (Hammett, 1936; Hammet and Steele, 1938). Guanine and arginine are alike in possessing a potential



configuration. No other nucleic acid component has this particular grouping. The tentative postulate is therefore advanced that the configuration noted above is a naturally occurring chemical factor concerned in the advancement of one or more of those preparatory processes which are essential for new growth production or Initiation.

It is interesting to observe that proliferation of recurrent growth is particularly forwarded by uracil, xanthine, thymine (Hammett and Lavine, 1937), cytosine, guanine, arginine, and allantoin; while neither hypoxanthine nor adenine have such effect. Those which forward this activity are alike in having an



grouping, or a carbon between two nitrogens which can attach to itself double-bonded oxygen or imide as an addition, substitution, or intramolecular rearrangement product of the configuration. The nucleic acid components and derivatives which do not have this property do not possess a carbon with this potentiality or situation. The nature of the valencies and substituents in positions 1, 2, and 3 of hypoxanthine and adenine are such that this part of the molecule cannot undergo intramolecular rearrangement so that the carbon of position 2 may add to itself double-bonded oxygen or imide. This of course is based on the assumption that the structural formulae given for

the several compounds under discussion are essentially true representations of the components and their relative positions.

Despite the fact that the advancement of recurrent growth proliferation is in every instance percentagely greater than the advancement of other expressions of proliferation, it cannot be claimed as a generalization that this is a result of advancement of recurrent growth initiation. It does not hold in every case. Thus with hypoxanthine there was enhancement of recurrent growth *initiation* but not of recurrent growth *proliferation*. Hypoxanthine advanced recurrent growth initiation because of oxygen in position 6. It did not advance recurrent growth proliferation because the carbon of position 2 could not take to itself double-bonded oxygen or imide. Further—in the case of cytosine there was no advancement of recurrent growth *initiation* but there was of recurrent growth *proliferation*. Recurrent growth initiation was inhibited by cytosine because of the NH_2 in position 6. Recurrent growth proliferation was advanced by cytosine because the carbon of position 2 could take on double-bonded oxygen. The evidence is indubitable that just as each of these two growth activities is the expression of individual and specific reactions, so has each its own separate and distinctive chemical group as a determinative factor. In the one case the carbon of position 6 and its associates, in the other the carbon of position 2 and its potentialities.

So far guanine has run true to form. Its influence on maintenance, on initiation as a whole, on initiation of recurrent growth, and on proliferation of recurrent growth is the same as that produced when the same chemical groups in the same configurations are present in other naturally occurring compounds.

The coincidence is evidence that the allocations so far made of definite chemical groupings to definite growth activities are veridical.

Outside of recurrent growth there was no marked general enhancement of proliferation expression. It therefore cannot be surely claimed that the compound acted as a stimulus to proliferation as a developmental activity.

It is a fact that the developmental activity of organization is advanced by guanine. It is a fact that this cannot be attributed to any maintenance reaction (Hammett, 1938). It is a fact that the same influence is exhibited by adenine, and a drift in the same direction by uracil. It should be noted that organization may be advanced more in the gonophores than in the hydranths. This appears in the uracil but not in the adenine data. Arginine on the other hand forwards gonophore organization but not that of hydranths. It is possible that two factors are here being exhibited. One for gonophores and one for hydranths. It is probable however that there is somewhere in this constellation a common derivative specifically essential to some process concerned in organization as a whole. What this is is of course unknown but I venture to predict that it will be found allied to an imidine grouping. Such as is conceivably derivable from combinations of positions 1, 2, and 3 or positions 1 and 6. Such as is potentially present in such precursors or derivatives of the pyrimidines as guanidine and divicine.

This discussion started out by indicating the probability that guanine intermediary metabolism is more through allantoin than through pyrimidine and

urea. Supplementary evidence is available. It is probable that xanthine breaks down through pyrimidine and urea rather than through allantoin (Hammett and Porter, 1938). If guanine followed the same course and if it first went to xanthine as Kiesel (1911) states, then it might be expected that the growth and metabolic reaction would in some considerable degree simulate that produced by its first product. But such is not the case. Where there is concordance this is traceable directly to specific chemical groups which are also present in other nucleic acid components and cause them to produce like effects. Outside of these there is no parallelism at all. Differentiation is essentially unaffected by guanine, what hint of reaction there may be is towards advancement. Xanthine on the other hand acts as a deterrent of this developmental activity. Guanine acts to forward organization expression. Xanthine acts to inhibit it. Regression is increased by xanthine and held back by guanine. Thus there is no parallelism and no evidence that guanine goes to xanthine in obelia intermediary metabolism.

No other nucleic acid component or derivative and no amino acid so far investigated produces a combined growth and metabolic reaction like that produced by guanine. The influence of guanine is specific and peculiar to this compound. Once more the postulate that each naturally occurring tissue component of general distribution has its own specific function in developmental growth is sustained by experiment.

SUMMARY AND CONCLUSION

Seventy-three experiments with close to 38,000 animals show that guanine acts to forward the developmental activities of Initiation and Organization in *Obelia geniculata*. It has no definite effect on Differentiation expression. Proliferation if it is affected at all is very slightly advanced. Maintenance of complete feeding hydranths is sustained and Regression thereof held back. Catabolism is advanced a bit. The compound may be toxic in certain concentrations. It acts specifically to advance initiation and proliferation of recurrent growths.

The picture is set by the individual character of the compound with respect to its peculiar combination of chemically effective groupings and intermediary metabolism by-products. The data confirm and sustain previous conclusions regarding specific growth functions of specific chemical constellations. They confirm allocation to position 6 of a role in recurrent growth *initiation*. They confirm allocation of separate participation in recurrent growth *proliferation* to the 1-2-3 configuration when the carbon thereof is able to add on double-bonded oxygen or imide. And they confirm the allocation of a maintenance sustaining and regression retarding property to imidazole.

They indicate that the 1-2-3 configuration is a specific forwarding factor for the developmental activity of Initiation as a whole when imide is attached to the carbon in position 2. They allow the tentative suggestion that a specific factor for Organization advancement may be found in the imidine configuration or some derivative thereof as a by-product of guanine and adenine intermediary

metabolism. And they allow the conclusion that the intermediary metabolism of guanine is through allantoin rather than pyrimidine and urea.

The work was done at The Marine Experimental Station of The Institute, North Truro, Massachusetts, with aid from Miss A. Bein of Philadelphia and the technical assistance of Misses Steele, Comee, and Hyde, and Messrs. Toman and Rivard.

REFERENCES

ALLEN, F. W. and L. R. CERECEDO. 1933. Purine metabolism. 11. The fate of guanine in the organism of the dog. *J. Biol. Chem.* **102,** 313–316.

COPPIN, N. S. S. 1913. Effects of purine derivatives and other organic compounds on growth and cell division in plants. *Biochem. J.* **6,** 416–421.

GUHA, B. C. and P. N. CHAKRAPORTY. 1934. Photochemical activation of adenine. Nature, **132,** 447.

HAMMETT, F. S. and N. CHATALBASH. 1935. Developmental growth and the amino acids. III. d-Arginine. *Protoplasma*, **23,** 492–502.

HAMMETT, F. S. 1936. Developmental growth and nucleic acid components. I. Uracil. *Biodynamica*, No. 9, 1–12.

HAMMETT, F. S. and M. L. ELLIOTT. 1936. Developmental growth and nucleic acid components. III. Allantoin. *Biodynamica*, No. 16, 1–4.

HAMMETT, F. S. T. LAVINE, and M. LAVINE. 1937. Developmental growth and nucleic acid components. V. Cytosine. GROWTH, **1,** 189–193.

HAMMETT, F. S., and T., LAVINE. 1937. Developmental growth and nucleic acid components. GROWTH, **1,** 135–139.

HANNETT, F. S. 1938. Developmental growth and nucleic acid components. VI. Adenine. GROWTH, **2,** 54–61.

HAMMETT, F. S. and M. R. PORTER. 1938. Developmental growth and nucleic acid components. VIII. Xanthine. GROWTH, **2,** 71–80.

HAMMETT, F. S. and T. STEELE. 1938. Developmental growth and nucleic acid components. VII. Hypoxanthine. GROWTH, **2,** 63–70.

KIESEL, A. 1911. Behavior of nucleic bases in plants kept in the dark. *Z. Physiol. Chem.* **67,** 241–250.

LEVENE, P. A. and L. W. BASS. 1931. Nucleic Acids. Chem. Catalog. Co. New York.

MENDEL, L. B. and T. SAIKI. 1908. Chemical studies on growth. IV. Changes in the purine, pentose, and cholesterol content of the developing egg. *Am. J. Physiol.* **21,** 77.

SAIKI, T. 1910. A study of the chemistry of cancer. II. Purine bases, creatinine and creatine. *J. Biol. Chem.* **7,** 23–26.

WELLS, H. G. and H. J. CORPER, 1910. The purines and purine metabolism in the human fetus and placenta. *J. Biol. Chem.* **6,** 469–482.

WELLS, H. G. 1912. The purines and purine enzymes of tumors. *J. Chem. Soc.* **102,** 371.

WELLS, H. G. 1913. The purines and purine metabolism of tumors and the chemical relation of primary and secondary tumors. *Z. Krebsforschung*, **12,** 598–611.

BOOK REVIEWS

THE PHYSIOLOGY OF PLANTS. Dr. William Seifriz. John Wiley and Sons, Inc. New York City. 1938.

It is with very mixed feelings that this reviewer attempts to gather together his impressions of Dr. Seifriz' book. Since it is written for college undergraduates, it must not be judged by the standards of a professional handbook. But, since it is written for students of botany who presumably would not be taking a second or third year course unless really interested in the subject, it must be judged as a botanical text and not as a mere cultural commentary. It must, therefore, be judged on the basis of simplicity, lucidity, and conciseness on the one hand and accuracy and comprehensiveness on the other.

Simplicity it certainly does have. The text flows easily and the presentation is well calculated to arouse the interest of the beginning student, in a variety of problems. On that score it can be highly recommended. Its lucidity and conciseness are very uneven. The chapter on permeability seems excellent (one would expect that from Dr. Seifriz), the one on salt requirements rather muddy, and the one on water relations (which the present reviewer helped to write!) still rather a tangle. So it is with other chapters. It is difficult to see the justification for devoting nine pages to the theory of acidity, thirteen pages to the colloidal state (both of them subjects an understanding of which is essential for any plant physiologist but which might better be learned in collateral reading), and allotting only fourteen lines to the subject of chemotropism, one of the most important single reactions of all living things. It is difficult to see why six pages should be devoted to the spiral habit, symmetry and harmony, four pages to the living and non-living, and only twenty-five lines to vegetative reproduction, which is the basis of much of our economic botany and involves some of the most fundamental problems in water relations, respiration, "hormone" action, permeability, and other processes to be found anywhere in the whole field of plant physiology. And, a propos this last subject, it is hard to see why seven of these twenty-five lines should be devoted to vegetative reproduction of moss protonema which the average student may never see and not a word to the potato, strawberry, dahlia, daffodil, willow, or any other important vegetatively reproduced plant within the student's everyday ken. This last criticism characterizes the basis of the general impression left by the book. One is left with the feeling that Dr. Seifriz has been so interested in dealing with ideas and processes that he has forgotten all about the plant. He seems to have reversed the proverbial process so that he "cannot see the trees for the forest." This is certainly no worse than the process repeated in so many other texts, of presenting the student with a compendium of facts in so dry a form that he cannot possibly swallow it. But one could wish that the author might have avoided both errors, so that the facts and ideas might have been better mixed in the same volume.

For college undergraduates the book has considerable merit, but it can hardly be said to really fill the existing need.

PHILIP R. WHITE

THE CULTURE OF ORGANS. Alexis Carrel and Charles A. Lindbergh. Paul B. Hoeber. New York City. 1938.

This is an extremely interesting and stimulating book which anyone concerned with the techniques of animal study might profitably read. Mr. Lindbergh has

described the construction, assembly, and working of his very ingenious apparatus in a lucid and concise manner which does credit to his engineering background. Dr. Carrel has done equally well by the portions dealing with the techniques of handling the organs involved, their manipulation, and the anatomical changes which such organs undergo during their cultivation *in vitro*. These are fields in which both authors are unquestionably masters.

When it comes to the physiological portions of the book as distinct from the technical, anatomical, and histological parts, the authors are obviously on much less firm ground. To take two examples which particularly caught this reviewer's attention:

The basis of most of the "artificial" nutrients used in these studies has been the ancient Tyrode solution (which, if the reader may have forgotten, was originally developed not as a nutrient but as a protective solution without published details as to the effects of its separate ingredients; see Tyrode, M. V. "The mode of action of some purgative salts." Arch. Intern. Pharmacodyn. Therapie 20: 205–223. 1910.). Naturally, one wishes to know the concentrations of the various ingredients employed in any given culture. In trying to find the concentration of, let us say, Ca in Solution E, one finds the following sequence. One starts with 400 ml. Ringer solution diluted with an indeterminant amount of ox-blood. To this are added 22.2 ml. double strength Tyrode solution, 547 ml. single strength Tyrode solution, and 30.8 ml. of other ingredients free of calcium. To 62.5 ml. of the above mixture are added 5.8 ml. double strength Tyrode, 1.5 ml. Ringer solution, and 165.56 ml. single strength Tyrode, each as solvent for some one or more calcium-free ingredient. Problem: to calculate the final calcium concentration. Ignoring the Ringer's solution, we have, if my mathematics is right,

$$\left[\frac{62.5}{250}\times\left(\frac{22.2}{1000}\times 2+\frac{547}{1000}\right)+\frac{5.8\times 2}{250}+\frac{165.56}{250}\right]\times C_{\text{Ca-Tyrode}}!$$

This solves out to be almost exactly one quarter the concentration of unmodified Tyrode solution or 18 mg. Ca per liter of nutrient, but who would have suspected it! One wonders if all this hocus-pocus is necessary! This is amusing rather than serious.

A more serious criticism, however, arises in considering the use of some of the particular ingredients, for example, insulin. Solution D contains approximately 1 unit insulin per liter of nutrient. The volume of fluid used in a single apparatus varies from 250 to 800 ml. and the volume of tissue from 0.1 to 1.0 g. If we take 500 ml. nutrient and 0.1 g. tissue, we are thus feeding 5 units of insulin per gram of tissue. A human being of 65 kilos weight would, on a comparable basis, receive 325,000 units of insulin! I submit to my diabetic friends who take insulin in doses of 20 or 40 or 60 units that the apparently beneficial effect of a dose of 325,000 units could hardly be due to the *hormonal* activity of this constituent! Such a quantity is easily sufficient to act as a nitrogenous nutrient. If one calculated the dosage on the basis of blood volume instead of body weight, the result would, of course, be less fantastic, but would still be excessive. Where dosages are given by weight instead of by "units," the concentrations are a bit easier to compare. Thyroxine at 0.1 mg. per liter of nutrient is a high but not excessive dosage, nor is 0.06 mg. riboflavin excessive. One is surprised to find no figures on concentration of thiamin (vitamin B_1), although its use is mentioned in the discussion of nutrients B and C.

Altogether, in spite of the emphasis which is laid on physiological problems in both the introduction and conclusions of the book, that part of the experimental portion which deals with the physiological aspects of the work so far carried out is certainly the weakest part. Here is an extraordinarily promising technique, developed and perfected by two men who are masters in their own fields, of surgery, anatomy and histology and of mechanics. Let us hope that it does not, as the tissue culture technique seems to have done, now fall into the doldrums of mere methodology for want of equal acuity on the side of physiology and physiological chemistry.

PHILIP R. WHITE

BOOKS ON NUTRITION WITH CHAPTERS ON GROWTH

CHEMISTRY OF FOOD AND NUTRITION. Henry C. Sherman. New York: The Macmillan Co. 1938. $3.25. Pp. 640.

THE FOUNDATION OF NUTRITION. Mary Swartz Rose. New York: The Macmillan Co. 1938. $3.50. Pp. 625.

FEEDS AND FEEDING. F. B. Morrison. Ithaca, New York: The Morrison Publishing Co. 1936. $5.00. Pp. 1050.

ANIMAL NUTRITION. Leonard A. Maynard. New York: McGraw-Hill Book Co., Inc. 1937. $4.00. Pp. 483.

These books by distinguished investigators and teachers reflect the contemporary knowledge of nutrition as taught in our colleges. They all contain good chapters on growth. Indeed, the nutritive values of foods are measured almost entirely by their influence on growth so that students of nutrition are necessarily students of growth at least from the nutritional viewpoint.

The book by Sherman was first published in 1911, and is now in the 5th edition. The book by Mrs. Rose was first published in 1927, and is now in the 3rd edition. The book by Morrison, now in its 20th edition, was first published by William A. Henry in 1898, and gradually expanded and republished first by Henry, then by Henry and Morrison, and now by Morrison. Maynard's book is in its first edition.

Sherman's purpose "is to present the principles of the chemistry of food and nutrition both as an integral part of the study of chemistry and with reference to the food requirements of man and the considerations which should underlie our judgment of the nutritive values of foods and the choice and use of food for the advancement of positive health. . . . The reader is especially urged to keep in mind the importance of a well-balanced knowledge of all the main factors of nutritive requirement—energy, protein, mineral elements, and vitamins." This book is very popular with college students majoring in science and nutrition.

The book by Mrs. Rose, lighter in style, "is written for those who wish to live more intelligently. An effort has been made to present within a small space some of the fundamental principles of human nutrition in terms which call for no highly specialized training in those natural sciences upon which the science of nutrition rests." This book is very popular with college students in home economics.

Morrison's book is agriculturally professional in outlook and substance. Its purpose is "to present . . . the most important facts concerning the feeding, care, and management of the various classes of larger farm animals. Also full information is given concerning the composition, use, and value of the many different feeding stuffs." Its unique feature is an extensive compilation of chemical compositions,

digestibilities, and net-energy values of feeding stuffs. These compilations are extremely helpful for estimating rations and their composition.

Maynard's book is addressed to students of nutrition of farm animals in Agricultural Colleges. It is assumed that the student has a knowledge of elementary organic chemistry and physiology. Comparative biologists will be most interested in Maynard's book because, unlike the books by Professors Sherman and Rose, it is not concerned primarily with human nutrition, but with the nutrition of the several species of farm animals, and it is therefore necessarily comparative in outlook. There is a tremendous literature on the nutrition of farm animals which Maynard has integrated successfully.

Students of growth, especially from the nutritional viewpoint, will find all these books very useful.

SAMUEL BRODY

INDEX TO VOLUME II OF GROWTH, 1938

Adaptive Enzymes, 363
Adenine, 55
African elephant, 335
Amino acid localization, 223

Biophysics, 213
Body Proportions, 1
Body Surface Area, 303
Body Weights, 197, 335
Bone, 327, 369
Book Reviews, 283, 397, 399
Brain Growth, 247
Brain Potentials, 247
Brody, S., 400

Callinectes sapidus, moulting in, 285
Carrel, A., 397
Cartilage, 327, 369
Cell Growth, 297
Cellular Forms, 213
Chapman, S. S., 223, 297
Chick Embryo, 261
Chick Embryos, normal stages, 145
Child Growth, 93
Colchicine, 257
Comee, R., 375

Dawson, W. M., 319
Dearborn, W. F., 197
Deming, W. E., 319
Dental caries, 149
Developmental Growth, 55, 63, 71, 347, 375, 389
Dewar, M. M., 13
Diffusion Forces, 165
d-Valine, 375
Dufrenoy, J., 159

Egg Weight, 267
Elephant, 335
Engel, G. C., 279, 381
Enzymes, 363
Estimation of Surface Area, 303

Fowl, 135, 141, 267
Frog Development, 25

Gesell, A., 283
Gray, E. H., 235, 285
Growth Equations, 247
Growth Stimulation, 355
Guanine, 389
Guinea Pig, 327, 369
Gunns, C. A., 261

Hamilton, B., 13
Hammett, F. S., 55, 63, 71, 223, 283, 297, 347, 375, 389
Havas, L. J., 257
Henderson, E. W., 267
Hoadley, L., 25
Human Body Surface, 303
Human Ovulation, 381
Human Tubal Ova, 381
Hypoxanthine, 63

Identification Chick Stages, 145
Increase of Population, 319
Infant Growth, 1
Inorganic Implantation, 49
Iob, V., 253

Klein, H., 149
Kramer, M., 149
Krauss, B. H., 181

l-Serine, 347
Lerner, I. M., 135, 141, 261
Lindbergh, C. A., 397

Mathematical Biophysics, 213
Metabolizing Systems, 165
Miller, B. J., 381
Mineral Growth, 253
Mitosis, 297
Moulting in Crabs, 285

Natural Increase, 319
Neural Induction, 49
Newcombe, C. L., 235, 285
Nucleic Acids, 55, 63, 71
Nutrition, 399

Obelia, 55, 63, 71, 223, 347, 375, 389
Okada, Y. K., 49
Organ and Body Weights, 335
Organ Culture, 397
Ova, 381
Ovulatory Time, 381
Owen, S. E., 355

Palmer, C. E., 149
Periodicity, 81
Phaseolus vulgaris, 297
Phytohormone, 257, 283
Pineapple Growth, 181
Plant Physiology, 283, 397
Population Increase, 319
Porter, M. R., 71
Potassium Iodide, 369
Psychology of Growth, 283

Quiring, D. P., 335

Rahn, O., 363
Rana pipiens, 25

Rashevsky, N., 213
Rat, 13, 253
Regeneration, 347
Reimann, S. P., 381
Relative Growth, 135, 141, 261, 235, 285
Retrogressive Growth, 327, 369
Rivard, D., 347
Roots, 159, 297
Rothney, J. W. M., 197

Seifriz, W., 397
Sideris, C. P., 181
Silberberg, M. and R., 327, 369
Simmons, K., 93
Stature and Weight, 93, 197
Steele, T., 63
Sulfhydryl, 297, 355
Sulphur, 355
Sulphuretted water, 159
Swanson, W. W., 253

Tarsometatarsus, 135, 141
Temperature and Development, 25, 261
Thimann, K. V., 283
Thompson, H., 1, 283
Thyroid Feeding, 327
Todd, T. W., 93
Tooth Age Determination, 149

Ungulates, 335

Valatx, M., 159

Walker, P. A., 145
Weight and Stature, 93, 197
Weinbach, A. P., 247, 303
Weinberg, A. M., 81
Went, F. W., 283
Wet and Dry Body Content, 13
White, P. R., 284, 397

Xanthine, 71

Young, G. E., 165

Papers may be sent directly to the editor representing the line of interest. In the case of rejection appeal may be made to the council. **Acceptable work will be published promptly as preprints with no restrictions as to length. To accomplish this papers must be accompanied by a minimum order for 200 preprints at $3.50 per page.** Separate rates for the first 100 preprints will be supplied on request.

All papers must be accompanied by an abstract not exceeding 225 words, and by a running title not in excess of 35 letters. Papers may be written in English, French, German, or Italian.

References should be listed alphabetically in the order: author, year, title, source, volume, paging. In the text references should be to the author and year, or by number. Summary and conclusions should be included; footnotes should be avoided. Changes from copy will be charged to the author.

The Auxiliary Publication Service of the American Documentation Institute at Washington is available for the publication, as micro-film or enlarged microphotographs, of excessive tabular or illustrative material. The Institute will also serve as a central deposit for original data.

Manuscripts and drawings should be submitted in complete and finished form with the authors' complete address. All drawings should be marked with the authors' name. Figures should be numbered and the reduction desired should be clearly indicated. Manuscripts should be typewritten in double spacing on one side of paper $8\frac{1}{2} \times 11$ inches, and should be packed flat. The original copy should be sent. Carbon copies cannot be considered. Original drawings when possible, should accompany the manuscript. Drawings intended for reproduction as line engravings are preferred and should be on blue-white paper or bristol board. Drawings should be accompanied by legends complete in themselves. For various methods of illustrating, authors are recommended to consult the Wistar Institute Style Brief.

All inquiries concerning publication should be sent to the *Managing-Editor*, Professor John Berrill, McGill University, Montreal.

Notice. The above rate of $3.50 per page represents a reduction of 50 cents per page. As rapidly as circumstances permit this charge for reprints will be further reduced.

CONTENTS

Paper		Page
64	GRAY, E. H. and C. L. NEWCOMBE.—Studies of Moulting in *Callinectes sapidus* Rathbun	285
65	HAMMETT, F. S. and S. S. CHAPMAN.—A Correlation between Sulfhydryl, Mitosis, and Cell Growth in Length in Roots of Phaseolus vulgaris	297
66	WEINBACH, A. P.—A Simple Method for Estimating the Surface Area of the Human Body from Birth to Maturity	303
67	DAWSON, W. M. and W. E. DEMING.—On a Problem of Natural Increase	319
68	SILBERBERG, M. and R. SILBERBERG.—The Effects of Thyroid Feeding on Growth Processes and Retrogressive Changes in Bone and Cartilage of the Immature Guinea Pig	327
69	QUIRING, D. P.—A Comparison of Certain Gland, Organ and Body Weights in Some African Ungulates and the African Elephant	335
70	HAMMETT, F. S. and D. RIVARD.—Developmental Growth and l-Serine	347
71	OWEN, S. E.—Sulphur and Growth Stimulation	355
72	RAHN, O.—On the Nature of Adaptive Enzymes	363
73	SILBERBERG, M. and R. SILBERBERG.—The Growth and Retrogressive Changes in Cartilage and Bone of the Guinea Pig Produced by Potassium Iodide	369
74	HAMMETT, F. S. and R. COMEE.—d-Valine in Developmental Growth	375
75	MILLER, B. J., G. C. ENGEL and S. P. REIMANN.—Studies of Unfertilized Human Tubal Ova. No. 2: Anatomic Observations of Three with Remarks on Ovulatory Time	381
76	HAMMETT, F. S.—Guanine in Developmental Growth	389
BOOK REVIEWS		397
INDEX		401

GEORGE BANTA PUBLISHING COMPANY, MENASHA, WISCONSIN